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Joel Ormala

ONE MORE STEP BACK – IMPURITY PROFILING AMPHETAMINE MADE FROM BENZYL CYANIDE AND PHENYLACETIC ACID

Master's thesis for the degree of Master of Science in Technology, submitted for inspection in Espoo, 3rd of October, 2016.

Supervisor **Professor Ari Koskinen**

Instructor **Dr. Pekka Joensuu**

Instructor **M. Sc. Sami Huhtala**

Aalto University
School of Chemical
Technology

Aalto University, P.O. BOX 11000, 00076 AALTO
www.aalto.fi

Abstract of master's thesis

Author Joel Ormala

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Abstract

The aim of this theses was to employ a multi-disciplinary approach to chemical impurity profiling of illicitly produced amphetamine.

Based on the literature on the illicit amphetamine market in Finland and Europe, the globally seized amphetamine pre- and pre-precursors, and the most commonly used illicit manufacturing methods, the synthetic routes to be studied in this research were determined. The role of chemical impurity profiling of drugs in intelligence-led policing strategies was explored, and the particular requirements of analytical methods employed in service of the forensic intelligence process were identified. Using this background knowledge, permission to conduct research on internationally controlled substances was applied and received from FIMEA.

Amphetamine was synthesized from benzyl cyanide and phenylacetic acid, using the Leuckart reaction and reductive amination. The amphetamine crude oil samples obtained by these four synthetic routes were analyzed, using a UPLC-TOF-MS method and a GC-MS method. Three route-specific impurities were tentatively identified, with the UPLC-TOF-MS method: 1,3-diphenyl-2-aminopropanone was only found in the two samples made from phenylacetic acid, 4,6-dimethyl-3,5-diphenylpyridine-2-one was only found in the two samples made from benzyl cyanide and 1-phenyl-2-propanol was only found in the sample made from benzyl cyanide, using reductive amination. The presence of 1-phenyl-2-propanol in the sample made from benzyl cyanide, using reductive amination, and the presence of 1,3-diphenyl-2-aminopropanone in the sample made from phenylacetic acid, using reductive amination were confirmed with the GC-MS method.

The preliminary findings presented in this study should be considered as the first part of a two-stage biased non-target screening process. In the second part of this process the results will be confirmed, by synthesizing and purchasing reference standards for the interesting impurities.

This thesis clearly demonstrates the benefit of a multi-disciplinary approach to impurity profiling synthetic drugs. By employing a two-stage screening process, starting with a rough and rapid analysis for detection, followed by a longer, more rigorous analysis for confirmation, the particular requirements of the forensic intelligence process have been taken into account. How route-specific impurities could be utilized in the forensic intelligence process was also proposed.

Keywords amphetamine, benzyl cyanide, phenylacetic acid, Leuckart reaction, reductive amination, impurity profile, forensic intelligence, illicit drug manufacturing, UPLC-TOF, GC-MS, drug screening

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Kemian tekniikan korkeakoulu

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www.aalto.fi
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Tiivistelmä

Diplomityön tavoitteena oli hyödyntää moni-tieteellistä lähestymistapaa amfetamiinin kemialliseen epäpuhtaus profilointiin.

Tässä työssä hyödynnettiin kirjallisuutta, joka käsitteli laittomia amfetamiini-markkinoita Suomessa ja Euroopassa, maailman laajuisesti takavarikoituja amfetamiinin pre- ja prekursoreita, sekä yleisempiä laittomia valmistusmenetelmiä. Kyseisen materiaalin perusteella valittiin kokeellisen osan synteesireitit. Kemiallisen epäpuhtaus profiloinnin roolia tiedustelu-lähtöisessä poliisityössä tutkittiin, ja rikosteknisen tiedustelun erityistarpeet käytettyjä analyysimenetelmiä kohtaan tunnistettiin. Hyödyntämällä tätä taustatietoa, FIMEA:lta haettiin ja saatiin lupa tutkita kansainvälisesti valvottuja aineita.

Amfetamiinia syntetisoitiin bentsyyli syanidista ja fenyylietikkahaposta, käyttämällä Leuckart reaktiota ja pelkistävää aminointia. Saatua amfetamiini öljyä analysoitiin UPLC-TOF ja GC-MS menetelmillä. Kolme synteesireitti-spesifiä epäpuhtautta tunnistettiin alustavasti: 1,3-difenyyli-2-aminopropanoni löydettiin vain fenyylietikkahaposta tehdyistä näytteistä, 3,5-difenyylipyridiini-2-oni löydettiin vain bentsyyli syanidista tehdyistä näytteistä ja 1-fenyyli-2-propanoli löydettiin vain bentsyyli syanidista pelkistävällä aminoinnilla tehdyistä näytteistä. GC-MS menetelmällä vahvistettiin 1-fenyyli-2-propanoli bentsyyli syanidista pelkistävällä aminoinnilla tehdystä näytteestä ja 1,3-difenyyli-2-aminopropanoni fenyylietikkahaposta Leuckart reaktiolla tehdystä näytteestä.

Näitä alustavia tuloksia tulisi tulkita kaksi-vaiheisen valikoivan ei-kohdennetun huumeseulonnan ensimmäisenä vaiheena. Seulonnan toisessa vaiheessa tulokset vahvistetaan valmistamalla tai ostamalla referenssi standardeja kiinnostaville epäpuhtauksille.

Tämä diplomityö osoittaa selvästi moni-tieteellisen lähestymistavan hyödyt synteettisten huumeiden epäpuhtausprofiloinnissa. Rikosteknisen tiedustelun erityispiirteet otettiin huomioon käyttämällä kaksi-vaiheista huumausaineseulontaa, jossa ensimmäinen vaiheen tarkoitus on löytää mahdollisia epäpuhtauksia, ja toisen vaiheen tarkoitus on varmistaa tulosten oikeellisuus. Työssä laadittiin myös ehdotus siitä, miten reitti-spesifejä epäpuhtauksia voitaisiin hyödyntää rikosteknisessä tiedusteluprosessissa.

Avainsanat amfetamiini, bentsyyli syanidi, fenyylietikka happo, Leuckart reaktio, pelkistävä aminointi, epäpuhtaus profilointi, rikostekninen tiedustelu, huumausaineen laiton valmistus, UPLC-TOF, GC-MS, huumeiden seulonta

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1 INTRODUCTION

Illegal drug trade is the world's largest illicit market, worldwide seizures of amphetamine-type stimulants (ATS) is growing faster than any other kind of illicit recreational drug[1] and amphetamine in particular is the most used ATS in Finland. Amphetamine is also the second most common illicit drug in Finland, after cannabis.[2] Legislation on precursors and pre-precursors is tightening, especially in the EU, forcing criminal organizations to deploy distributed and global manufacturing networks that utilize longer synthetic pathways than before.[3–5] In other words, the need and difficulty to identify the origin of seized synthetic drugs is growing fast.

However, current methods for profiling impurities in synthetic drugs have been developed 10-15 years ago and many of them lack indicators for reliable identification of starting materials in multi-step synthetic pathways.[6] At the same time, budgetary constraints have decreased the research contribution of public forensic laboratories leaving current synthetic drug profiling methods in need of updating. Therefore, there is an increasing demand for academic research, in collaboration with forensic laboratories, to enable law-enforcement agencies to have up-to-date forensic methodologies.

The most common precursor of amphetamine seized in Europe is phenyl-2-propanone (P2P). The two most common methods for transforming P2P to amphetamine are the Leuckart reaction and reductive amination. Currently chemical profiling methods focus on identifying immediate precursors as well as other impurities related to amphetamine synthesis. However, as P2P has been included in the list of Table 1 controlled precursors in 1988 illicit manufacturers are taking one more step back and make P2P by themselves, from either phenylacetic acid or alpha-phenylacetoacetonitrile (APAAN).[7] In 2009, 2010 and 2011 global seizures of phenyl acetic acid were significantly higher than P2P seizures. However, legislation followed suite and in 2011 phenylacetic acid was made a Table 1 controlled precursor. In 2012 global seizures of P2P were again higher than phenylacetic acid seizures.[8] Since 2011 APAAN has emerged as the most seized non-controlled pre-precursor to amphetamine in Europe, until in 2014 it was also made a Table 1 internationally controlled precursor. Benzyl cyanide, a precursor to APAAN has also been seized in relation to P2P manufacturing in Mexico[9] and there is a growing suspicion that illicit manufacturers are starting to make APAAN by themselves from benzyl cyanide.

In the present study amphetamine was synthesized from phenylacetic acid and benzyl cyanide by two different synthetic methods; the Leuckart reaction and reductive Al/Cl_2Hg-amination, in order to obtain impurity profiles for all four synthetic routes. Based on the literature, two hypotheses about route-specific impurities were made: 1) alpha-benzylphenethylamineformamide would be found in amphetamine made from phenylacetic acid via the Leuckart reaction, but not in amphetamine made from benzyl cyanide.[6] 2) 4,6-dimethyl-3,5-diphenylpyridine-2-one would be found in amphetamine made from benzyl cyanide via the Leuckart reaction, but not in amphetamine made from phenylacetic acid.[10] To understand how these route specific impurities would be utilized, if found, the role of chemical impurity profiling in intelligence-led policing strategies was explored, and the particular requirements of analytical methods employed in service of the forensic intelligence process was identified.

In order to conduct academic research on controlled substances the Finnish National Bureau of Investigation's forensic laboratory was approached with a collaboration proposal. It was agreed that the required synthesis would be made in the Laboratory of Organic Chemistry at Aalto University and that the products would be analyzed in the forensic laboratory, with their current GC-MS profiling method, in addition to the UPLC-TOF analysis in Aalto University. In addition, the necessary permissions from the Finnish Medicine Agency (FIMEA) were sought and received. The entire process, from collaboration negotiations to permission applications, synthesis and impurity profiling was completed between September 2015 and October 2016 as part of this thesis, leading to the Master of Science and Engineering degree.

2 INTERNATIONALLY CONTROLLED SUBSTANCES – CONVENTIONS, LAWS AND REGULATORY BODIES

2.1 THE INTERNATIONAL DRUG CONTROL MECHANISM

"Pharmakon" is an ancient Greek word and it can be translated to "remedy" or "poison." This double meaning and function is true for most of our known pharmacologically active substances. Therefore governments, authorities and the international community have simultaneously set restrictions to prevent abuse, while allowing indispensable pharmaceutical and scientific use of these "pharmakons." It is with these two crucial, but competing needs in mind that the international drug control mechanism was constructed.[11]

2.1.1 THE INTERNATIONAL DRUG CONTROL CONVENTIONS

There are three primary international drug control conventions today: 1) The Single Convention on Narcotic Drugs from 1961[12], 2) The Convention on Psychotropic Substances from 1971[13] and 3) United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances from 1988.[14]

The Single Convention on Narcotic Drugs was established to streamline the multitude of existing conventions under one umbrella. The convention focuses mainly on natural products derived from opium poppy, coca bush leaves and the cannabis plant. The list of controlled substances under the convention has grown considerably since 1961 and today the convention covers more than 120 substances. Parties of the convention have agreed to restrict the production, manufacturing, export, import, distribution, trade and possession of the controlled substances with the aim of minimizing their abuse.[15]

The Convention on Psychotropic Substances from 1971 is similar to the 1961 convention in its control mechanism, but it concerns psychotropic substances, such as central nervous-system stimulants, sedative-hypnotics and hallucinogens. Currently 130 substances are controlled under this convention.[16]

The United Nations convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances in 1988 focuses on information sharing, co-operation and harmonization between signatory parties. It defines jurisdiction, right of confiscation, obligations of extradition, information sharing and mutual legal assistance between the signatory states.

Significantly, it also adds substances frequently used in the manufacturing of controlled substances (as defined in the two earlier conventions) to the list of internationally controlled substances.[17] These precursors are classified in Table 1 and Table 2 substances. Table 1 substances have more rigorous regulations than Table 2 substances. P2P was a Table 1 precursor in the original 1988 convention, Phenylacetic acid was transferred from a Table 2 pre-precursor to a Table 1 pre-precursor in 2011 and APAAN was made a Table 1 pre-precursor in 2014.

2.1.2 International Drug Control Bodies

The International Narcotics Control Board (INCB) is defined as an independent monitoring body in all three international drug control conventions. Its function is to monitor the implementation of the drug control conventions in the signatory states. To do so, it administers a statistical system to estimate the licit use, and balance between supply and demand, of controlled substances in signatory states. It also facilities and analyzes information provided by the signatory states about the amount and quality of seized substances under international control, as well as substances used for their manufacturing, both controlled and not controlled. Based on this information the INCB makes recommendations of the inclusion or exclusion of substances from the list of internationally controlled substances (as defined by the three conventions). The INCB releases annual reports on these functions.[18]

The Commission on Narcotic Drugs (CND) is a commission established under the United Nations Economic and Social Council to review and analyze the global drug situation, considering interrelated issues of prevention of drug abuse, rehabilitation of drug abusers, as well as trafficking in illicit drugs. Based on the recommendations of the INCB and the World Health Organization (WHO), which is responsible for assessing the health implications of new and potentially harmful drugs, the CND has the authority to place substances of abuse and their precursors on the list of internationally controlled substances. The CND is also responsible for evaluating and changing the category of internationally controlled substances. This process is referred to as scheduling. In addition to its normative function, the CND also has an operational role as the governing body of the United Nations Office of Drugs and Crime (UNODC), which provides expertise to member states, as well as to the CND and INCB in all matters related to the world drug problem.[19]

2.2 European Union Regulations on Internationally Controlled Substances

The European Union (EU) follows the international drug control conventions, but adds to them by two regulations (approved by both the European Parliament and the European Council, based on the European Commission's proposal) and one Council Decision (made by the European Council alone, based on the European Commission's proposal). The first regulation, EC 273/2004 defines additional classes of precursors and regulations for their trade inside the EU. The second regulation, EC 111/2005 lays down rules of monitoring the trade of the precursors defined in EC 273/2004 with non-EU countries. The council decision 2005/387/JHA gives the European Council the authority to place substances of abuse under national control within the EU, as if they would be controlled under the 1961 and 1971 UN conventions. The council used this authority in 2005 to place benzylpiperazine and mephedrone under pan-European control.[20]

The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) was established in 1993 to provide expertise in all drug related matters both to the EU and its organizations as well as to its member states, akin to the UNODC.

2.3 Finnish Laws, Decrees and Regulatory Bodies Related to Drug Control

The international drug conventions and the EU regulations and decisions related to drug control have been ratified in Finnish legislation. The Finnish law governing controlled substances is called "Huumausainelaki" (373/2008). It defines how controlled substances are legally handled, traded and manufactured, what obligations institutions dealing with controlled substances have and which body is responsible for the implementation of the law. The law is supplemented with decrees, namely decree 543/2008, which lists the controlled narcotics and drugs related to 373/2008 3 § 2 moment (based on EC 273/2005 and UN 1961 convention). Decree 1130/2014, in turn, lists the controlled psychotropic substances related to 373/2008 3 § 3 moment (based on EC 111/2005 and the UN 1971 convention). The controlled substances listed in decree 543/2008 and 1130/2014 are regularly updated. The latest updates are from January 2015, by decree 61/2015 and decree 62/2015 respectively. Decree 548/2008 specifies required permissions for various legal activities related to controlled substances. The criminal offences of illegally handling, producing, selling and trafficking internationally controlled substances are defined in the Finnish criminal law (39/1889) chapter 50 1-8 §.

Finnish legislation does not recognize generic or analogous classes of controlled substances, which means that every new controlled substance (however chemically close to an already controlled substance), has to be individually named and added to decree 543/2008 or 1130/2014. This ensures strong protection for Art. 7(1) of the European Convention on Human Rights, "No one shall be held guilty of any criminal offence on account of any act or omission which did not constitute a criminal offence under national or international law at the time when it was committed." However, it also means that even the slightest chemical variation to a controlled substance can make it legal, even if the pharmacological effects are the same, or the intention is to transform it back to a controlled substance. This is frequently exploited by criminal organizations.[21]

Finland does not have emergency procedures for temporarily placing a legal substance under national control or rapid procedures to accelerate the normal process of placing substances under national control. Instead, Finland brings new substances under national control only after UN and EU decisions.[21]

The Finnish Medicine Agency (FIMEA) is the governing and monitoring body responsible for the implementation of law 373/2008. It also grants permissions for the various legal activities defined in decree 548/2008 and ensures that sufficient precautions are taken by everyone handling controlled substances.

3 AMPHETAMINE AS AN ILLICIT RECREATIONAL DRUG

3.1 BRIEF GLOBAL HISTORY

Amphetamine is believed to have been first synthesized in 1887 by Romanian chemist Lazar Edeleanu. However, it was only in the 1930ies its pharmacological properties and medicinal use gathered more interest. It was then sold over the counter as a medicine against low blood pressure, narcolepsy, ADHD, depression and obesity, in at least the United States, the United Kingdoms, Netherlands, Germany and Sweden. In the Second World War amphetamine was widely distributed to soldiers of all sides to increase performance and endurance. Partly due to the common usage during the war and the large stocks left after it, in the 1950ies amphetamine use became widely accepted among the general population and medical professionals alike. However, as the usage grew, amphetamine's addictive properties also became better known. Consequently, in the 50-ties amphetamine became a prescription drug in the United States and much of Europe. That did not decrease its consumption and from 1958 to 1970 the legal production of amphetamine increased from 3,5 billion tablets to 10 billion tablets per year in the United States alone. Amphetamine became an integral part of the 1960ies social movement and it was estimated that during that time more than half of the legally produced amphetamine in the United States ended up on the black market. However, it was only after amphetamine was made an internationally controlled substance in 1971 that large-scale illicit manufacturing of amphetamine started both in the United States and Europe. As a consequence, amphetamine usage and manufacturing also became more tied to criminal organizations, like various biker gangs in the United States. [3,22]

During the 1960ies methamphetamine began to replace amphetamine as the ATS of choice globally and today it is by far the most used ATS worldwide. Global methamphetamine seizures in 2014 were 108 tons and global amphetamine seizures were 48 tons.[23] However, the exception to this global trend is Europe, especially northern Europe, where amphetamine is the most frequently used ATS. European amphetamine seizures in 2013 were 8.2 tones and methamphetamine seizures 0.8 tones.[24] In 2014 the corresponding figures were 7.4 tons and 0.8 tons respectively.[4] As opposed to the global methamphetamine market, were the manufacturing location and the end market are frequently on different continents, amphetamine is predominantly produced locally in Europe for the European market and in the Middle-East for the Middle-Eastern market.[3,23]

3.2 AMPHETAMINE IN FINLAND

Amphetamine usage in Finland during the 1930ies, 1940ies, 1950ies and 1960ies followed the global and European pattern, as described earlier.[25] After the war it was frequently used in homes to aid normal tasks, such as in cleaning, driving and building tasks. In the 1950ies and 1960ies different brand names were used for specialized amphetamine products, such as Lergigan (allergy medicine) and Adjudets (for obesity), as seen in Image 1. In 1968 amphetamine was criminalized in Finland and its medicinal usage ended. As in the United States, after criminalization amphetamine was no longer procured from legal medicine manufacturing, but from illicit manufacturing controlled by organized criminal networks.[26] Among the European countries, where it is possible to analyze longer term trends, Finland is the only one in which amphetamine usage has increased since 2000.[4] Combined amphetamine and methamphetamine seizures in Finland have remained relatively steady since 2003. Roughly 5-15 % of those seizures are methamphetamine and the rest is amphetamine.[2] When analyzing daily mean amounts of amphetamine found in wastewater per number of people in the population, Antwerp has the highest concentration of amphetamine, with around 250 mg/1000 people/day. Helsinki shares second place with Oslo and Berlin, with around 100 mg/1000 people/day.[4] In most other European countries, cocaine is the second most used drug after cannabis derived substances, but in Finland it is amphetamine.[2,4]

Image 1. Brand names for amphetamine based medicine sold in Finland in the 1950ies and 1960ies.

3.3 Illicit Amphetamine Manufacturing & Trafficking in Europe

3.3.1 Common Illicit Synthetic Routes to Amphetamine

There are literally hundreds of different routes to synthesize amphetamine[27], but only a few of them are commonly used in illicit manufacturing.[3] The Leuckart reaction is one of the most common reactions in amphetamine synthesis. The reaction starts from P2P, which is used as an alkylating agent of an amine, with an acid acting as a reducing agent. It is therefore called a *reductive alkylation* reaction. The reaction was first described by Leuckart in 1885, who used ammonium formate and formamide as the amine source. In 1893 Wallach improved upon the reaction, using ammonium formate in the presence of excess formic acid. While technically different reactions, they are both called the Leuckart reaction in the literature and that convention will be followed here, too. Studies on the mechanism of the reaction have been published in 1944[28], 1948[29] and as late as 1999[30]. In the reaction, formylamphetamine is produced as an intermediate, which is then hydrolysed with an acid to produce the corresponding amphetamine.[6]

Another way to convert P2P to amphetamine is through *reductive amination*.[31] In it an amine (ammonia, in the case of amphetamine) is used to form a hemiaminal species with P2P that is then converted to an intermediate imine. A reducing agent, such as cyanoborohydride, sodium borohydride or aluminium is then used with a mercury chloride catalyst to reduce the imine to amphetamine.[6]

Another commonly used starting material for illicit amphetamine manufacturing is l-norephedrine and its stereoisomer d-norpseudoephedrine. Both of these isomers can be reduced to amphetamine by a number of methods. The "Nagai" method uses hydrodic acid and red phosphorus[32], the "Moscow" method uses iodine and red phosphorous[33], and the "Hypo" method uses iodine and either hypophosphorous acid or phosphopric acid. These reactions are stereoselective and are capable of producing only the active d-isomer of amphetamine.[6]

Another way to convert l-norephidrine or d-norpseudoephidrine to amphetamine is through the radical Birch reaction.[34] In it metallic lithium or metallic sodium is used in the presence of liquid ammonium to create a radical anion of norephedrine or norpseudoephedrine, which through the loss of the hydroxyl group and by gaining another electron is converted to a carbanion and then further to amphetamine. Metallic lithium is more commonly used, because it is easily available in lithium batteries.[6]

The Emde method also starts with l-norephedrine or d-norpseudoephedrine, but it is a two-step reaction. In it thionyl chloride is used to create a chloro substituted intermediate, which is then reduced with hydrogen in the presence of a catalyst, such as palladium or barium sulfate.[6]

It has also been reported that benzaldehyde is used together with an acid or base to produce a nitrostyrene intermediate, which is then reduced with electrolysis, hydrogenation or other reducing agents (such as lithium aluminium hydride or sodium borohydride) to give amphetamine.[6] The most common synthetic methods to manufacture amphetamine are summarized in Image 2. Regardless of the synthetic route used, the produced amphetamine base (also called amphetamine oil) is finally converted into amphetamine sulfate, a whitish water soluble powder, consumed by the end user.[3]

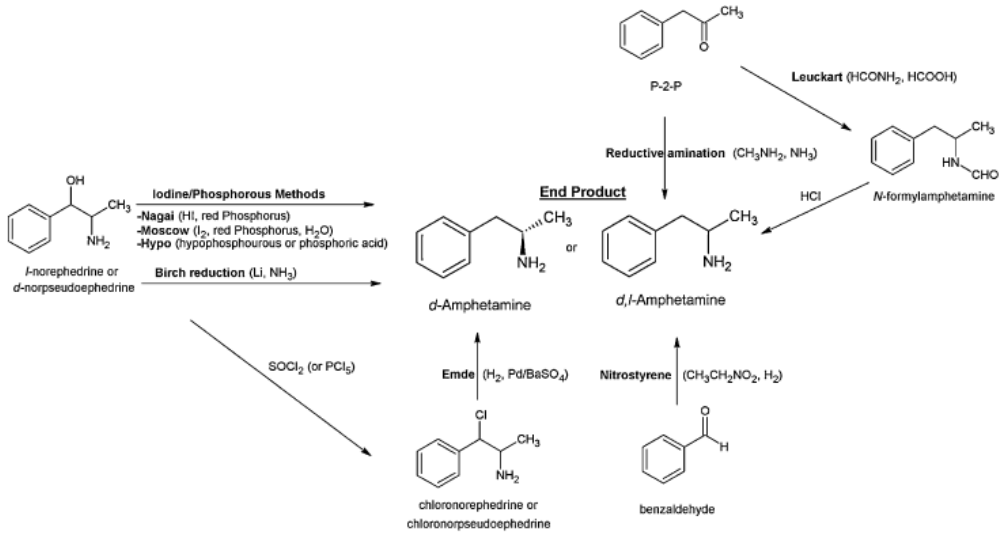


Fig. 3. Common synthetic routes of AP manufacture.

Image 2. Common illicit synthetic routes to make amphetamine.[6]

3.3.2 Illicit Amphetamine Manufacturing in Europe

Historically, illicit amphetamine in Europe has been synthesized from P2P. In the United States and Asia the precursor has been either d-norephedrine or l-norpseudoephedrine, which is legally produced in the medical industry.[6,8,9] However, a recent trend in the United States and Asia shows an increase in amphetamine and methamphetamine produced from P2P. [9] In 2011 around half of the global P2P seizures originated in Europe, but in 2012 that decreased to 12%.[8] Until 2004 the origin of P2P ending up on the black market in Europe was almost exclusively from legitimate production in China. However, between 2005 and 2009 forensic profiling indicated a change. During that time, the majority of P2P seized on the black market in Europe originated from legitimate production in Russia. From 2009 onwards the amount of self-produced P2P has increased in Europe[3] and in 2014 and 2015 most of the globally seized P2P were reported to originate from illicit self-manufacturing.[9]

Illicit P2P is most commonly made from either phenylacetic acid or APAAN, but also benzaldehyde has been reported as a precursors.[9] In 2009 - 2011 phenyl acetic acid seizures were significantly higher than P2P seizures in Europe, but the situation was reversed in 2012 after phenylacetic acid was rescheduled a Table 1 controlled substance under the 1988 UN convention.[8] To convert phenylacetic acid to P2P either lead acetic

acid or acetic anhydride is used.[6] The reaction between phenylacetic acid and acetic anhydride is also called the Dakin West-reaction.[35]

In 2013 APAAN seizures in Europe rose to 43,5 tons, while in 2014 the corresponding number decrease to 11 tons, still the most seized precursor to amphetamine by far. The largest single seizure of APAAN, 5.1 tons was made in Germany in 2014 and it originated from China.[4,9] APAAN is hydrolyzed to P2P using a strong acid (such as concentrated sulfuric acid) and high temperature. A seizure of amphetamine that was shown to have been made from APAAN also contained benzyl cyanide.[36] APAAN can be made from benzyl cyanide, benzaldehyde and phenylacetic acid.[37]

The most commonly reported non-scheduled pre-precursor to amphetamine seized in Europe was in 2014 benzaldehyde, but the total amount was just 12 kg.[9]

3.3.3 Illicit Amphetamine Trafficking in Europe

The illicit amphetamine market in Europe is highly lucrative, with an estimated retail price of up to 300% over the wholesale price. [3] The mean retail price of amphetamine in Europe ranged from 9.5 to 30 €/g in 2014, with the lowest prices being found in Belgium and the highest in Scandinavia and Malta. The mean purity of seized amphetamine in Europe ranged from 10.3 to 48.9 % in 2014, with the lowest purity being found in Bulgaria and the highest in the Netherlands followed by Sweden, Norway and Belgium. The mean purity of amphetamine seized in Finland in 2014 was 24%.[38]

Most amphetamine consumed in Europe is also manufactured in Europe. The manufacturing of amphetamine is concentrated into regional criminal hubs. The largest of these hubs is concentrated around the Netherlands and Belgium and together they account for most clandestine amphetamine manufacturing in Europe. The North Western hub also has the largest and most professional production facilities, capable of producing between 20 and 50 kg of amphetamine per day. The primary market of the North Western hub is the United Kingdom and Scandinavia. The second largest concentration of amphetamine manufacturing in Europe is the North Easter hub, which includes Poland and the Baltic countries. The primary markets for the North Eastern hub is local consumption in the area, as well as exportation to Scandinavia, often through Estonia to Finland, and to the Middle-East through Turkey.[3,5] Recently Bulgaria has been reported as a significant illicit amphetamine producer as well.[5]

The majority of amphetamine seized in Finland is produced in western Europe, in the North Western hub, although it is trafficked either through Sweden or Estonia.[2] However, another report suggests that much of the Amphetamine available in Finland is manufactured in the North Eastern hub.[5]

To summarize, compared to P2P an increasing amount of seizures of phenylacetic acid and APAAN are being reported. At the same time an increasing amount of amphetamine is seized as a base (oil) and not as amphetamine sulfate (powder).[4] Considering these facts there seems to be a trend of more dispersed production networks, where pre-precursors, precursor and amphetamine synthesis, as well as crystallization of amphetamine into amphetamine sulfate are all done in different places, to decrease the risk of being caught trafficking an easily identifiable internationally controlled substance. Another trend seems to be the utilization of longer synthetic routes to avoid handling and trafficking of controlled precursors. Indeed, the most recent EMCDDA report on amphetamine production in Europe suggests that the number of production facilities is decreasing, while their capacity and professionalism is increasing, both in the North Wester and in the North eastern hub. The report also suggests that most of the amphetamine produced in these hubs are today manufactured from APAAN.[5] This poses significant technical challenges to identifying current production and trafficking trends. As the INCB states, "[The] INCB encourages Governments that have the technical capabilities to conduct such detailed forensic analyses and offer such support, to the extent possible, to other Governments on request, with a view to improving knowledge of the chemicals actually being used in illicit drug manufacture and the sources of those chemicals, thus supporting precursor control measures worldwide."[9]

4 Drug Profiling – From Trace to Intelligence

4.1 Forensic Science in Service of Criminal Justice and Intelligence-Led Policing

The most basic function of forensic science is to say something about a past and unknown activity (like a crime), based on traces (anything observable or measurable) left behind. This need was born out of the criminal justice system, which needed "hard facts", preferably science based to supplement witness testimonies and other more "unreliable" or "subjective" evidence. However, it soon became clear that these traces were also useful during the criminal investigation of a committed crime, especially when combined with other, traditional investigative methods, like interrogations or phone records.[39] However, both the evidence evaluation and the investigative function of forensic science are reactive in the sense that they occur after a crime has been committed.

Reactive security and policing strategies have not been deemed the most cost-effective ones in a globalized world with organized crime. Consequently, these strategies have moved towards more proactive and intelligence-led frameworks, with an emphasis on crime prevention.[40,41] This is accomplished by studying the criminal activity holistically, beyond the individually committed crimes and beyond illegal activities, to provide information and intelligence to strategic decision-making. This information can prioritize resources towards the most critical and disruptive elements in the criminal activity.[42] For example, dismantling a clandestine amphetamine laboratory will decrease the amount of amphetamine on the illicit market much more than seizing some grams of amphetamine from the end user. Thus, intelligence-led policing can be described as a "conceptual framework for conducting the business of policing and a way to organize information that allows police agencies to better understand crime problems (in particular how, when and why crimes are occurring) and use resources in a proactive manner."[41]

Forensic intelligence is a term used to describe the third function of forensic science, in addition to its role of providing forensic evaluation of evidence to the justice system and aiding individual criminal investigations. This function provides timely information about the criminal activity as a whole in order to support decision-making in intelligence-led policing strategies.[43]

Another way to understand the function of forensic intelligence is to divide it into different levels: tactical, operational and strategic. *Tactical intelligence* is related to the

investigative function described earlier and it supports case specific actions, such as evidence gathering. *Operational intelligence* assists in planning crime reductions strategies, for example by identifying priorities in different geographical areas. *Strategic intelligence* provides an understanding of wider patterns of criminal behavior and its environment. It is future-oriented and explores long-term solutions.[44] It should be noted, that the differences between different levels of intelligence is not clear cut, as often forensic evidence in a single case may contribute information to strategic intelligence as well.[41]

The forensic intelligence results should also be understood as something more than a simple reconstruction of an individual criminal event (as opposed to forensic evidence). The process of obtaining forensic intelligence can therefore be divided into three stages, as illustrated in Image 3. 1) The identification of data or traces, which has no meaning on its own, but serves as the foundation for interpretations. 2) Information or signs obtained from the traces that allow the reconstruction of a single event or to draw links between different events, like a statistical link between two drug profiles coming from two different drug seizures. 3) Synthesis of various sources of information, when interpreted and analyzed in the context of a specific question. The idea is to go beyond the information related to a single case to form a hypothesis about a previously unknown phenomenon, like a trend in drug trafficking patterns.[41,45]

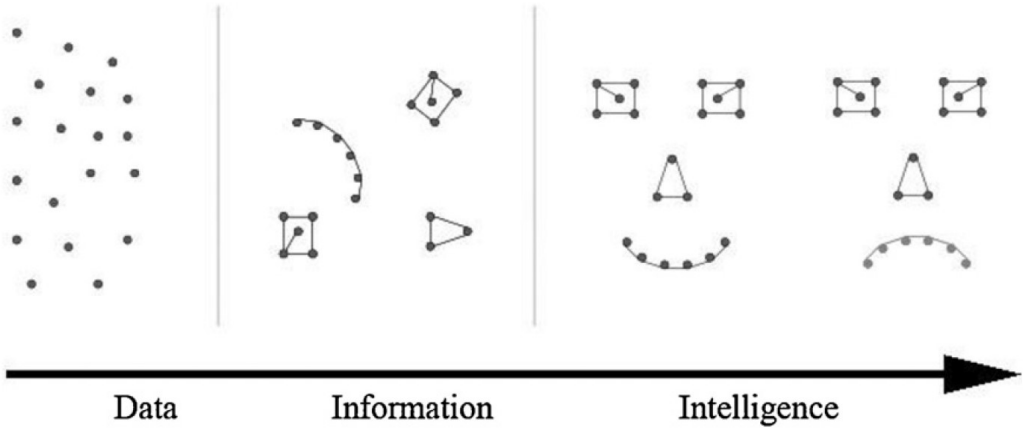


Image 3. The forensic intelligence process as illustrated by Cartier. [41,45]

4.2 Drug Profiling as a Tool in the Forensic Process

Drug profiling is a process in which chemical and/or physical characteristics of drugs are compiled into profiles, categorized into classes and used to provide evidence for the

judicial process or intelligence for various functions.[46] A *profile* in this context can be understood as a collection of traits, for example certain impurities found in a drug sample that are specifically chosen for the profiling process. A *class*, in turn, is a group of samples having similar profiles, when compared after statistical methods are applied to the output of the analytical method. If two drug seizures are found to have similar enough profiles, the profiles belong to the same class and the two drug seizures are said to be *linked*. The linking of seizures, using the sample profiles categorized into classes is illustrated in Image 4.[39]

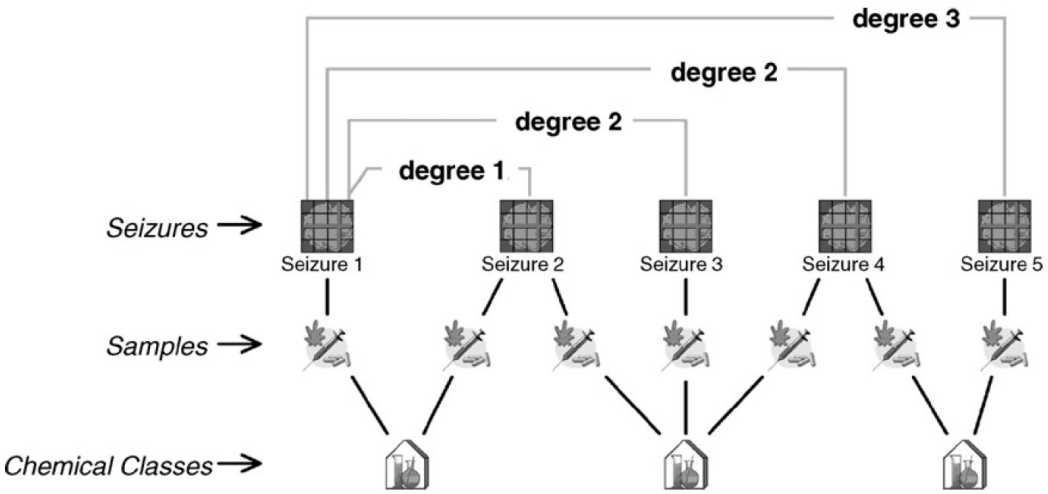


Image 4. How samples of seizures with a specific chemical profile are categorized into classes and linked through connections of varying degrees.[39]

For drug profiles to be useful beyond a single case they have to be stored in a database for future use. This allows new samples coming from later seizures to be compared to the entire database, not only to cases, which are known to be linked to the new seizure by other police information. This enables two important functions: a) the confirmation of a previously suspected link and b) orientation towards a previously unsuspected link.[39,40]

In summary, drug profiling can be said to address two different roles in the forensic process. 1) A more traditional role, in service of the judicial system and criminal investigation. For example, if several small drug seizures are linked to a common trafficker by their profiles, it can be used as evidence of the extent of his criminal activities and influence his sentence. Or, if a drug seizure is analyzed quickly enough, the characteristics of the profile can be used to direct the interrogation of the trafficker apprehended in relation to the seizure. 2) A broader role in service of forensic intelligence. For example,

identifying a change in manufacturing techniques and precursors used in illicit drug manufacturing can influence how international drug policy is decided and what precursors are placed under international control. How drug profiling contributes to the different functions in the forensic process is illustrated in Image 5.[41]

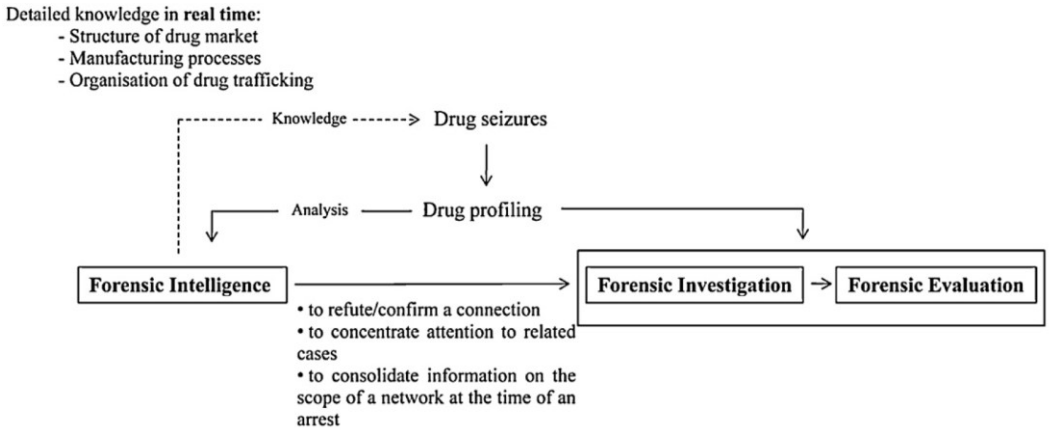


Image 5. How drug profiling contributes to different functions in the forensic process. [41]

While often similar, drug profiling in service of the intelligence process has sometimes different needs than drug profiling in service of the judicial system. One such significant difference is the importance of time versus accuracy. If an individual link between two profiles is to be used as evidence in a criminal trial, the certainty of that link needs to be relatively high in order to prevent miscarriage of justice. This is why the methodologies employed by forensic laboratories need to be thoroughly validated and constantly accredited. This in turn leads to new profiling methodologies being introduced into forensic laboratories relatively slowly. Time, on the other hand, is usually less of an issue, since the trial can be held only once the necessary investigation is complete. However, the situation is reversed when it comes to forensic intelligence, where time is often a critical factor. Accuracy does not have to be as high, because a drug profiling link used in the intelligence process is most often combined with other sources of intelligence to provide a relatively accurate picture overall, even if an individual intelligence source or result is somewhat uncertain. In other situations even an uncertain indication can be enough to guide further research or investigation in the right direction, since the intelligence process is iterative in nature.[39,43,46,47] Other differences between judicial and intelligence driven forensic science objectives are summarized in Table 1.[43]

Table 1. Differences between justice and intelligence driven forensic science. [43]

	Forensic science	
	Justice	Security
Underlying model	Criminal-law driven.	Intelligence-led.
Domain/methodology	Forensic investigation and evaluation.	Forensic intelligence.
Objectives	Bringing criminals to justice.	Devising most efficient strategies and concrete operations for disrupting crime activities.
Organisational settings	Separate the scientific settings (forensic laboratory) from police organisation. Independent laboratories.	Integrate information extracted from traces into crime intelligence.
Use of traces	Investigative leads and court decision based on evaluated and accepted evidence.	Feeding crime analysis in order to offer best quality information.
Risks of poor interpretation and deficient communication	Miscarriage of justice.	Incomplete and biaised understanding of the criminal environment that, in turn, lead to poor security decisions on strategies and operations.
Efficiency	Search for the best ratio cost/crime detection for each type of trace. Generally privileging the more individualising traces.	Efficient contribution to security aims, for instance crime reduction or the reduction of the fear of crime.

4.2.1 Statistical Drug Profiling Methods

To compare impurity profiles of drugs it is crucial that the used profiling method is similar among the compared profiles. Given that drug trafficking is an international problem a high degree of standardization and harmonization of profiling methodologies is needed to trace the origin of a seized drug. One such massive undertaking was the European project, "Collaborative Harmonization of Methods for Profiling of Amphetamine Type Stimulants" and the creation of a common database to store profiling information obtained by the methodology.[41,46] As a part of this program, The Harmonized Method for Profiling Amphetamine was developed as a collaboration between the National Forensic Laboratories of Finland and Sweden, as well as the University of Lausanne and the University of Strathclyde between 1999 and 2002.[48] The project resulted in six articles published between 2005 and 2007.

First, 21 reference standards for impurities found in seized amphetamine were synthesized and full analytical data was obtained for them.[49] Then the stability of the synthesized references in different organic solvents were investigated, in order to find the most inert and therefore the most suitable organic solvent for amphetamine profiling.[50]

Next, a chromatographic method was developed. Gas-chromatography (GC) was chosen, due to its good separation, high degree of stability (repeatability and reproducibility) and user-friendliness. Four aspects of the GC method were optimized: 1) the sample introduction technique (split or splitless injection at various temperatures), 2) the stationary phase and temperature gradient to achieve required chromatographic separation, 3) the used detector (flame ionization detector (FID), nitrogen phosphorous detector (NDP) and mass-spectroscopy (MS) in both scan and selected ion monitoring mode), 4) the volume of the injected sample, using amphetamine samples synthesized by

the Leuckart route, reductive amination or nitrostyre route. These four aspects were optimized by obtaining usual performance metrics: reproducibility, repeatability, linearity and limits of detection. The MS detector showed advantages compared to the other two detectors due to its unique selectivity.[51]

To extract impurities from amphetamine samples liquid-liquid extraction and solid-phase extraction were evaluated as sample pre-treatment methods.[52] Also the variability of the analytical method within and between laboratories was evaluated, using peak areas of the FID and MS detectors. Both detectors performed similarly. Day to day variation within one laboratory was between 8-10% and inter-laboratory variation was between 8-12 %, as given by the mean relative standard deviation of the peak areas of each compound.[53]

Different statistical methods for comparing the samples, based on the selected impurities were evaluated last. This was done in three main steps: 1) First different pretreatment methods were applied to the peak areas in the chromatogram (weighting and normalization, as well as using different logaritms and roots to reduce the influence of large peaks). 2) The ability of different pretreatment methods to separate profiles of amphetamine samples synthesized by different methods (Leuckart, reductive amination and nitrostyrene) was evaluated,[54] using a partial least squares discriminant analysis (PLS-DA),[55] on the peak areas of the selected impurities (as shown in Image 6). 3) The linked distance (comparing one sample to other samples from the *same* production batch or similar synthesis method) and unlinked distance (comparing one sample to other samples *not* from the same production batch or similar synthesis method) was evaluated, by comparing the ratio of the linked and unlinked distance produced by different numerical methods to measure distance. The best separation between samples was obtained by pretreating the data by normalization (to the sum of the areas of the selected target impurity compounds) and the 4^{th} root. The highest ratio between linked and unlinked distances was gained with the Pearson correlation.[54]

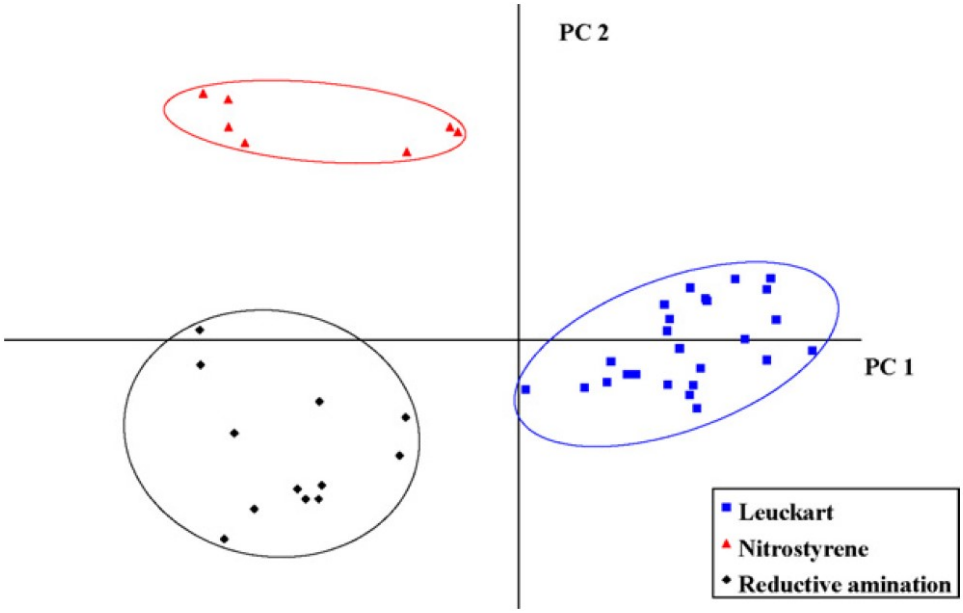


Image 6. PLS-DA performed on the peak areas, pre-treated by normalization and the 4th root, of target impurities in amphetamine, synthesized by the Leuckart, reductive amination and nitrostyrene method. The results show the best separation of the evaluated pre-treatment methods.[54]

Other similar statistical drug profiling methods have also provided convincing evidence of their ability to reliably link seizures originating from the same batch to each other. For example, the harmonized method for analyzing MDMA, also developed under the European harmonization program, found strong links between MDMA samples seized in Switzerland and the Netherlands, supporting the assumption that the MDMA was synthesized in the Netherlands and then distributed around Europe.[46] A similar study was conducted in Australia and similarly clear links between different MDMA seizures were found, but in addition, the links between different chemical profiles were validated, by comparing them to links found or suspected based on other policing methods.[47]

However, statistical comparisons of drug profiles have certain limitations, which were highlighted in a study by Morelato, et. al.[56] The study tried to link samples of different methamphetamine seizures made by the Australian Federal Police to each other, but they found that the samples taken from a single seizure showed so much variation in their profiles that it became difficult to link any other seizure to it. Different explanations were discussed; was it a sampling problem, or were the seizures composed of methamphetamine made by different people in different laboratories, or was it possibly

the same person and laboratory using different methods and starting materials? These questions can only be answered, if we know the reason for the variation of specific impurities (as opposed statistical variations in the whole impurity profile). Therefore, we must continue to synthesize controlled substances and study the chemistry that produces the impurities, in order to reach the full potential of chemical profiling.[56]

4.2.2 Route-Specific Impurities in Amphetamine

Route-specific impurities or *characteristic impurities* are compounds, which are formed only if a specific synthetic route or starting material is used to synthesize the target product (like amphetamine **1**). Route-specific impurities have been studied less than statistical profiling methods, since they cannot alone be used to link two drug seizures to each other. However, they can be used as marker compounds in statistical impurity profiling methods to extract additional information out of the profile, as discussed in the previous section. The primary method for studying and identifying route-specific impurities is by synthesizing controlled substances, using different methods and starting materials, and comparing individual impurities found in the synthesized batches.[6,10,57,58]

Stojanovska et. al. conducted a comprehensive review on the reported impurities related to ATS synthesis. They identified several potential route-specific impurities for methylamphetamine **2**, 3,4-methylenedioxymethylamphetamine, amphetamine **1**, dimethylamphetamine and p-methoxyamphetamine.

Curiously, only 1,3-diphenyl-2-aminopropane **3** and *alpha*-benzylphenethylamineformamide **4** are identified as related to a pre-precursor by Stojanovska et. al., all the other impurities found in amphetamine **1** are only traced back to an immediate precursor and the synthetic method used. These two impurities are formed, when the P2P **5** used in the amphetamine synthesis also contains 1,3-diphenyl-2-aminopropanone **6**.[6,59,60] **6** is formed as the minor product, in a 70:30 ration, when P2P **5** is made from phenylacetic acid **7**, using acetic anhydride **8**.[35,61] *Alpha*-benzylphenethylamineformamide **4** is identified as a route specific impurity for the Leuckart method, but 1,3-diphenyl-2-aminopropane **3** is formed also in the reductive amination.[6]

The only other potential route-specific impurity specific to a pre-precursor of amphetamine **1** that was found in the literature reviewed for this thesis is 4,6-dimethyl-

3,4-diphenylpyridine-2-one **9.**[10] It was reported by Power et. al. in relation to a seizure of amphetamine that was shown to have been made from P2P **5**, which in turn was made from APAAN **10**.[10,36]

The Leuckart reaction produces amphetamine **1** through the intermediate *N*-formylamphetamine **11**, which in the presence of formamide can undergo condensations to form a number of route specific hetero-cycles.[62] The two pyridine structures that have been confirmed are 2,6-dimethyl-3,5-diphenylpyridine **12b** and 2,4-dimethyl-3,5-diphenylpyridine **12a**.[63]

No route specific-impurities for amphetamine synthesized by reductive amination was reported by Stojanovskaja et. al.[6] However, *N*-acetylamphetamine **13** was reported in amphetamine **1** made by reductive amination, even if it was not considered route specific.[64] 1,5-diphenyl-4-methylpent-1-ene **14**, which decomposes to 1,5-diphenyl-2-methyl-4-oxopent-1-ene **15** has also been tentatively identified when amphetamine **1** is synthesized by reductive amination.[65]

However, for methamphetamine **2**, one route specific-impurity for reductive amination was reported, 1-phenyl-2-propanol **16**, which is formed when the starting ketone P2P **5** is reduced to the corresponding alcohol.[6] This was first reported by Verweij[64] and later confirmed by Kunalan et. al., when they compared methamphetamine **2** synthesized from P2P by the Leuckart reaction and reductive amination. They also noted that 1-phenyl-2-propanol **16** was only found in a mildly acidic (pH 6.0) extraction, but not in a basic (pH 10) extraction.[58] This is worth noting, because nothing should prevent 1-phenynl-propanol **16** from forming when synthesizing amphetamine in similar conditions. Indeed, its formation was indicated (but not confirmed) by Allen & Cantrell when excess ammonia is used in the reductive amination.[31]

As summarized by Stojanovska et. al., “Marker compounds are of most significance when accurately identifying the synthetic method; i.e. when they are route-specific or precursor-specific.”[6] The structures of potential route specific impurities, precursors and pre-precursors related to amphetamine **1** (and methamphetamine **2**) synthesis discussed above are shown in Image 7.

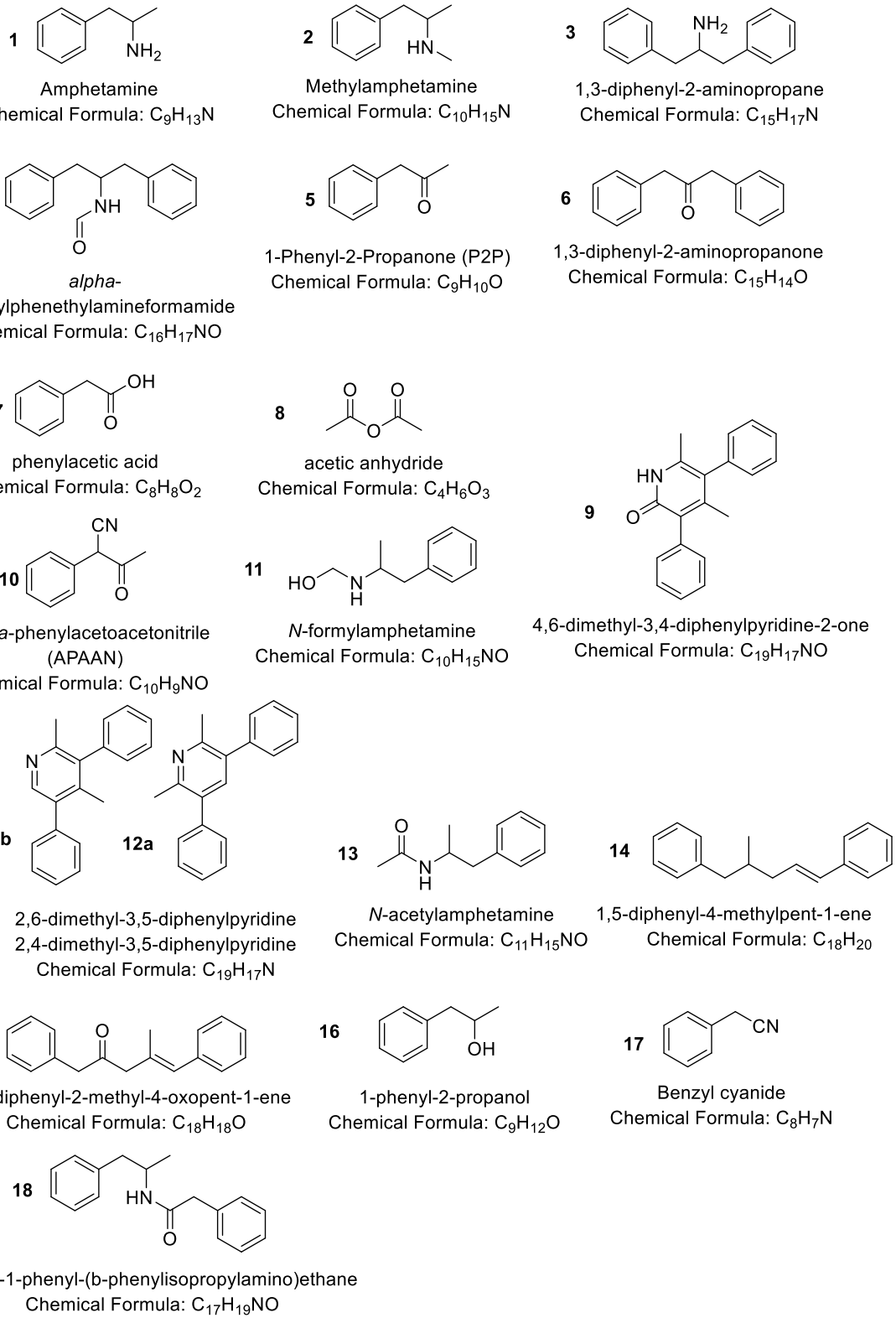


Image 7. Compounds used in the synthesis of amphetamine from phenylacetic acid and benzyl cyanide, and potentially interesting impurities.

4.3 Analytical Methods

Identifying controlled substances and their impurities in seized samples poses a number of challenges for the analytical method. Due to the requirements of the legal system the *selectivity* (ability to produce distinct responses for different substances) needs to be high. To accomplish this, the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), which is an American expert group consisting of forensic scientists from around the world, have recommended either a Category A technique (like MS) together with a Category B technique (GC or LC) or two uncorrelated Category B techniques (like HPLC-UV and GC-FID) for positive identification of controlled substances, when used in court.[66] UNODC gives a similar recommendation in their guide to Recommended Methods for the Identification and Analysis of Amphetamine, Methamphetamine and their Ring-Substituted Analogues in Seized Materials.[67]

The most common analytical method used in forensic laboratories for routine identification and in universities for research purpose is GC in combination with either a MS or FID detector. Especially, the GC-MS combination offers great chromatographic *separation* (the way chromatographic methods introduce selectivity), combined with a detector that is highly selective. It is therefore sometimes called a hyphenated method.[51,67,68]

Separation with all chromatographic methods is achieved by the interaction of a stationary phase, usually contained in a column, and the target compound, which is transferred through the column by a mobile phase. GC is a viable method only for volatile compounds with a relative low molecular mass and a high thermal stability, because the analyzed substances have to be vaporized without decomposition. This has proven to be particularly problematic with New Psychotropic Drugs (NPS), like synthetic cannabinoids. Sometimes these limitations can be overcome by derivatization, the process of transforming a chemical compound to another to make it more suitable for GC analysis, but that is a time-consuming technique. HPLC, on the other hand, does not suffer from these limitations. However, the separating power of HPLC is substantially lower compared to GC. The relatively poor separation of HPLC can be improved significantly with Ultra-High Performance Liquid Chromatography (UPLC), which uses columns packed with smaller particles that offer more stationary surface and smaller instrumental void volumes, thus causing higher operating pressures.[69] Good separation with any

chromatographic method used in combination with MS is important in order to reduce interference of overlapping fragments and over-saturation of the MS detector. This is a particular problem when analyzing samples containing many different compounds, like biological samples[70], modified synthetic drugs[71], or any other drug sample with a many impurities and a complex background matrix.

A mass spectrometer works by ionizing the analyzed compound in an ion source, so that it can be accelerated and separated, using an electric or magnetic field in a mass selector and finally detected by the charge or current induced by the ions. The electron-ionization (EI) technique, most commonly used together with GC, is a hard ionization technique, resulting in much fragmentation. EI is a widely used technique, which offers reproducible fragmentation that can be used to detect and identify substances with the help of mass spectral libraries. A high fragmentation rate can also be problematic, if the sample contains many substances, because of overlapping fragments. A softer GCMS ionization technique, like chemical ionization (CI), could be a better method in those cases, because it causes less fragmentation and offers some selectivity depending on the used reagent gas. Electron spray ionization (ESI) used with LCMS techniques could also be better in complex samples, because it, too, causes less fragmentation.[70] The strongest peak with this technique is usually the non-fragmented molecular ion plus a proton or natrium atom (designated $[M+H]^+$ and $[M+Na]^+$ respectively).[68]

Low-resolution mass spectrometers (LRMS), like ion-trap instruments and linear quadrupole mass filters only give molecular masses in whole numbers. Identification of the studied compound is achieved by its unique fragmentation pattern, but for reliable identification a reference standard is always needed. However, high-resolution mass spectroscopy (HRMS), like the time-of-flight (TOF) selector, gives molecular weights with an accuracy of up to four digits (ppm levels). It is therefore possible to identify molecular formulas based on their unique isotopic ratio with HRMS. This is particularly useful, when searching for substances without references, like previously unknown impurities in synthetic drugs. This is called *unbiased non-target screening*, when nothing is known about the substance and *biased non-target screening*, when something, like the molecular formula (and therefore exact mass) is known. Ibáñez, et. al. developed a comprehensive analytical strategy for the screening of NPS, using TOF HRMS in a multi-step process, starting with a tentative screening without references and only then confirming the

interesting findings with references.[71] Another significant advantage of non-target screening using TOF HRMS is the fact that reprocessing of the data and data mining techniques can be performed any time after the initial analysis.[71,72] Both of these qualities provide very interesting possibilities with regards to impurity profiling of synthetic drugs, especially from an intelligence-led perspective, as will be analyzed further in the discussion section 6.3.

5 RESEARCH WORK AND RESULTS

5.1 RESEARCH PROPOSAL AND FIMEA APPLICATION PROCESS

The present study was conducted on internationally controlled substances and therefore the University needed to apply for permits from FIMEA. In order to file the permit applications a detailed research proposal was needed to justify why and exactly how much controlled substances had to be handled and manufactured. This made the planning of the research challenging, since a normal iterative process, where the next experiment is decided based on the results from the previous experiments, could not be employed. Consequently, most of the literature presented in this thesis was reviewed well in advance of the experimental work. The literature review and the writing of the permit application was completed between September and December of 2015 in consultation with FIMEA and professor Jari Yli-Kauhaluoma from the University of Helsinki, who had participated in a similar application process for Helsinki University.

If the research on controlled substances is done in a specific Faculty, it is sufficient that the Faculty in questions applies for the permit. First a permission to handle controlled substances is needed (372/2008 § 15), which ensures that the organization has sufficient measures in place to handle controlled substances securely and prevent possible misuse. The permit application has to contain at least: 1) Information about the applying organization, 2) Information about the suitability of the applicant (as defined in 373/2008 11 §), 3) A presentation of the conducted activity and how the transportation, storage, destruction and record keeping is handled related to the controlled substance, 4) A description of the facilities in which the activity is conducted, their contact information and a description of the personnel and their access to the used facilities. 5) Information about the person(s) in charge of the activities related to the controlled substance (as required in 373/2008 16-17 §), including: a) their full name and social security number, b) their contact information, c) their responsibilities in the organization, d) their expertise in handling the controlled substance and e) attestation that they are not in bankruptcy and that they are not under custody. In addition, information about the handled controlled substance, its amount and its intended purpose is needed. Each application can only include one controlled substance, so separate applications have to be filed for each handled controlled substance.

The present study required that each controlled substance was synthesized, and consequently a manufacturing permit (as defined by 373/2008 12 §) for each substance was also needed. A manufacturing permit can only be given to an organization with a handling permit. However, the handling permit application and the manufacturing permit application can be filed simultaneously. The application for the manufacturing permit has to include information about the controlled substance, the amounts manufactured and its intended purpose.

Both the handling and the manufacturing permit can be granted for no more than one calendar year at a time, but the permits can be renewed with premade forms found on the FIMEA webpage. In addition, FIMEA requires annual reports about the handled, manufactured and imported controlled substances, their amounts, purpose of usage and current stocks. The report has to be handed to FIMEA no later than the 15th of February the following year.

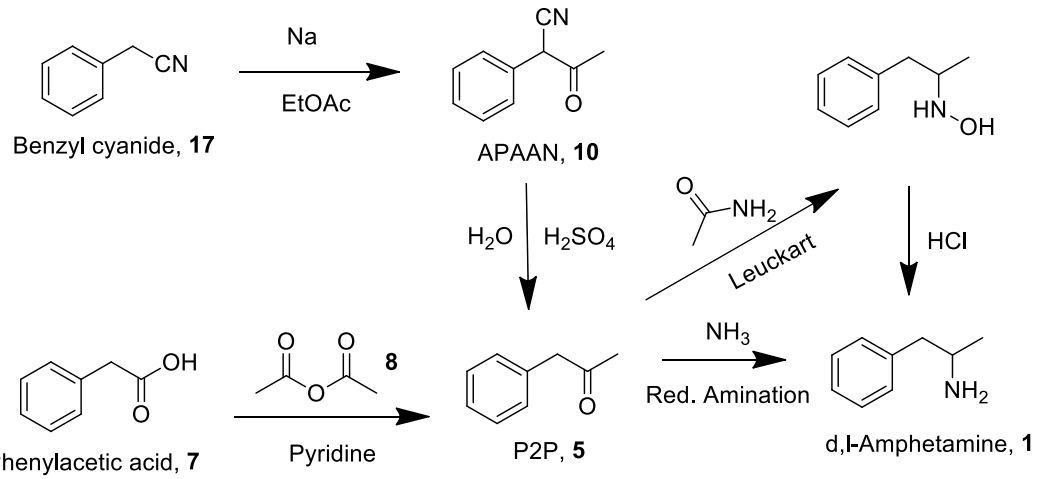
Necessary approvals from the Dean of the Faculty and people in charge of the controlled substances were obtained. The permit applications were sent to FIMEA on the 21st of December 2015. The censored handling permit application for amphetamine can be found as an example in Appendix 1. The censored manufacturing permit application for amphetamine can be found as an example in appendix 2.

In January 2016 FIMEA requested corrections to the document for the manufacturing permit of amphetamine, which erroneously contained the manufacturing permit application for phenylacetic acid instead of amphetamine. The correct document was sent to FIMEA instantly. A more extensive request for additional information of various elements in the applications was received from FIMEA in mid-February 2016. This request was responded to by supplying clarifications to the initial applications (see Appendix 3), with a comprehensive research plan (see Appendix 4) and a handling permit application for APAAN (identical to the one for phenylacetic acid and amphetamine) within a week. Another request for clarification was received from FIMEA in the beginning of March, which was answered by phone on the same day. FIMEA approved all submitted permit applications on the 3rd of March, finishing the application process after 73 days.

An additional manufacturing permit application for APAAN was sent to FIMEA in mid-March and it was approved two weeks later.

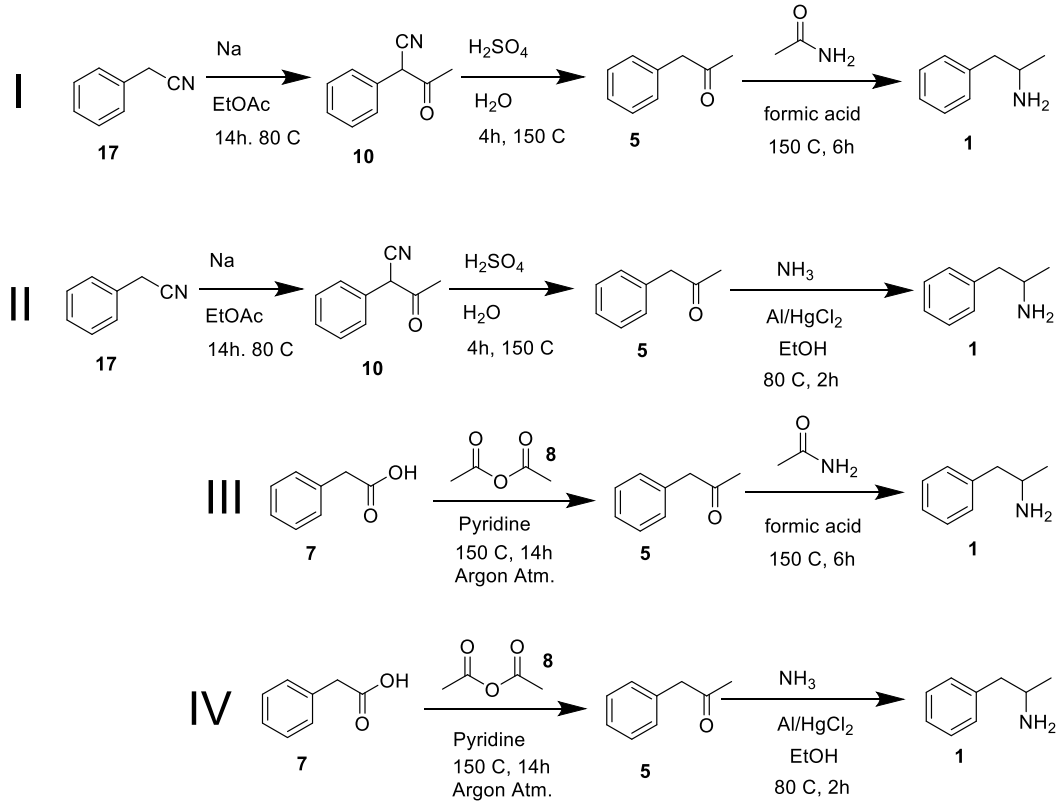
5.2 SYNTHESIS

Based on the literature reviewed for Chapter 2, the synthetic routes presented in Scheme 1 were chosen for this study. These were deemed the most relevant reactions to study further, in order to better understand the origin of amphetamine found on the black market in Finland - now and in the future.



Scheme 1. Overview of the studied synthetic routes to Amphetamine.

The studied synthetic routes can be divided into four (**I-IV**) distinct synthetic pathways, which are presented with key conditions in Scheme 2.



Scheme 2. The four (**I-IV**) studied synthetic pathways to amphetamine with key conditions.

In route **I** APAAN **10** is made from benzyl cyanide **17**, using metallic sodium.[73] APAAN **10** is then converted to P2P **5** using concentrated sulphuric acid.[36] Finally, P2P **5** is converted to amphetamine **1** by the Leuckart reaction, using formamide and formic acid.[36] Route **II** is similar to route **I**, except for the last step, in which P2P **5** is converted to amphetamine **I** by reductive amination, using an aluminium mercurychlorid amalgam and an excess of ammonia.[74]

In route **III** and **IV** P2P **5** is made from phenylacetic acid **7** and acetic anhydride **8**, using pyridine as catalyst in dry conditions.[35] In route **III** P2P **5** is converted to amphetamine **1** by the Leuckart reaction and in route **IV** by reductive amination.

The first reaction to be tried was the conversion of phenylacetic acid **7** to P2P **5**. It was first done on a small scale (giving 100-200 mg of crude P2P **5**) to ensure it worked. The reaction was first tried in room temperature, as it was the normal conditions presented in the reference[35], but no conversion was observed by thin-layer chromatography (TLC) or nuclear magnetic resonance (NMR). However, the supplementary information revealed

that when using pyridine as catalyst refluxing is needed. To obtain a pure P2P **5** reference several attempts to purify the crude P2P **5** by column chromatography were made. The H^1 and C^{13} NMR spectra for the successfully purified P2P **5** can be found in Appendix 7. Using these spectra as reference, the success of all the studied routes to produce P2P **5** was confirmed. However, in the actual research reactions neither P2P **5** or APAAN **10** were purified with column chromatography to mimic clandestine amphetamine **1** manufacturing. All the ^{1}H NMR spectra of unpurified P2P **5** made from phenylacetic acid **7** showed the presence of the starting material **7** and dibenzyl ketone **6.**

The conversion of P2P **5** to amphetamine **1** was first done on a small scale (giving 100-200 mg of crude amphetamine **1**) using the Leuckart reaction. The reaction proved to be difficult to monitor using TLC, as the spots were completely overlapping. The impurity of the crude product was confirmed by H^1 and C^{13} NMR. However, the presence of amphetamine **1** was tentatively confirmed by comparison to spectra found in the literature. Several attempts to purify the crude amphetamine **1** oil with Kugelrohr distillation were made, but the attempts improved the purity only slightly according to the NMR spectra.

The conversion of benzyl cyanide **17** to APAAN **10** and the conversion of **10** to P2P **5** posed no problems. The P2P **5** made from benzyl cyanide **17** was almost as clean as the purified P2P **5** according to NMR. Notably, the spectra did not contain any indication of dibenzyl ketone **6** observed in the P2P **5** made from phenylacetic acid **7.**

The reductive amination of P2P **2** was done using aluminium folio instead of aluminium grit to mimic clandestine manufacturing. The reaction worked well and produced a noticeably cleaner amphetamine **1** crude oil, both in route **II** and **IV**, compared to the Leuckart reactions in routes **I** and **III**, as judged by the NMR spectra. The amphetamine **1** oil from route **II** looked cleaner and was yellow, compared to the reddish porridge from route **IV.** The amphetamine **1** oil from route **I** was also clean looking and yellow, but the oil from route **III** was a very dark brown.

All the actual research reactions were scaled to produce between 1 and 1.5 g of crude amphetamine **1** oil. As the research interest in this study was the impurities, the main focus of the study was the amphetamine **1** oil and not the crystallized amphetamine salts. However, in order get an indication of the purity of crystallized amphetamine salts compared with amphetamine **1** oil, several crystallization attempts were made. The

amphetamine **1** oil from route **II** was crystallized to amphetamine chloride right after the synthesis and to amphetamine sulfate a week after synthesis. The amphetamine **1** oil from route **IV** was crystallized to both amphetamine chloride and amphetamine sulfate right after the synthesis. 151 mg of amphetamine **1** oil from route **II** yielded 116 mg of white and clean looking amphetamine sulfate crystals, as confirmed later by GC-MS analysis. 145 mg of crude amphetamine **1** oil from route **IV** yielded 73 mg of white and clean looking amphetamine sulfate crystals, as confirmed later by GC-MS analysis. This also indicates a higher purity for the amphetamine **1** made from benzyl cyanide **17** than from phenylacetic acid **7**. No NMR spectra were obtained for the amphetamine salts to leave enough substance for later chemical profiling.

5.3 UPLC-TOF-MS ANALYSIS

The crude amphetamine **1** oils obtained from route **I**, **II**, **III** and **IV** were analyzed using UPLC and TOF-MS parameters adapted from Nielsen et. al.[70] In order to explore the performance of the adapted parameters, attempts to verify the methodology were made. Several batches of amphetamine standard diluted into concentrations suitable for a calibration curve were made. The linear range for amphetamine quantification reported by Nielsen et. al. was 0.5 ng/ml – 2.5 ng /ml. A typical batch of amphetamine standards were therefore made with concentrations from 100 ng/ml to 0.1 ng/ml to explore the limit of detection (LOD) and limit of quantification (LOQ), as well as possible over-saturation of the detector at higher concentrations. The TOF-MS was calibrated in the beginning of each day experiments were run. Before each experiment-run an empty gradient run in the UPLC was also made. However, despite several attempts, reproducibility, technical reliability and accuracy of the methodology was poor. The total ionization current (TIC) chromatogram (when searching for the $[M+H]^+$ ion of amphetamine) for a typical experiment with amphetamine standards is shown in Image 8. The mass accuracy from concentrations 100 ng/ml to 5 ng/ml is shown in Table 2. The ppm error range for the measured amphetamine standards 1-5 was between -77.9 and 13.2 ppm and normalized i-FIT values varied between 0 and 3.9. Consequently, any attempts to properly verify the method or to make a semi-quantitative analysis was abandoned.

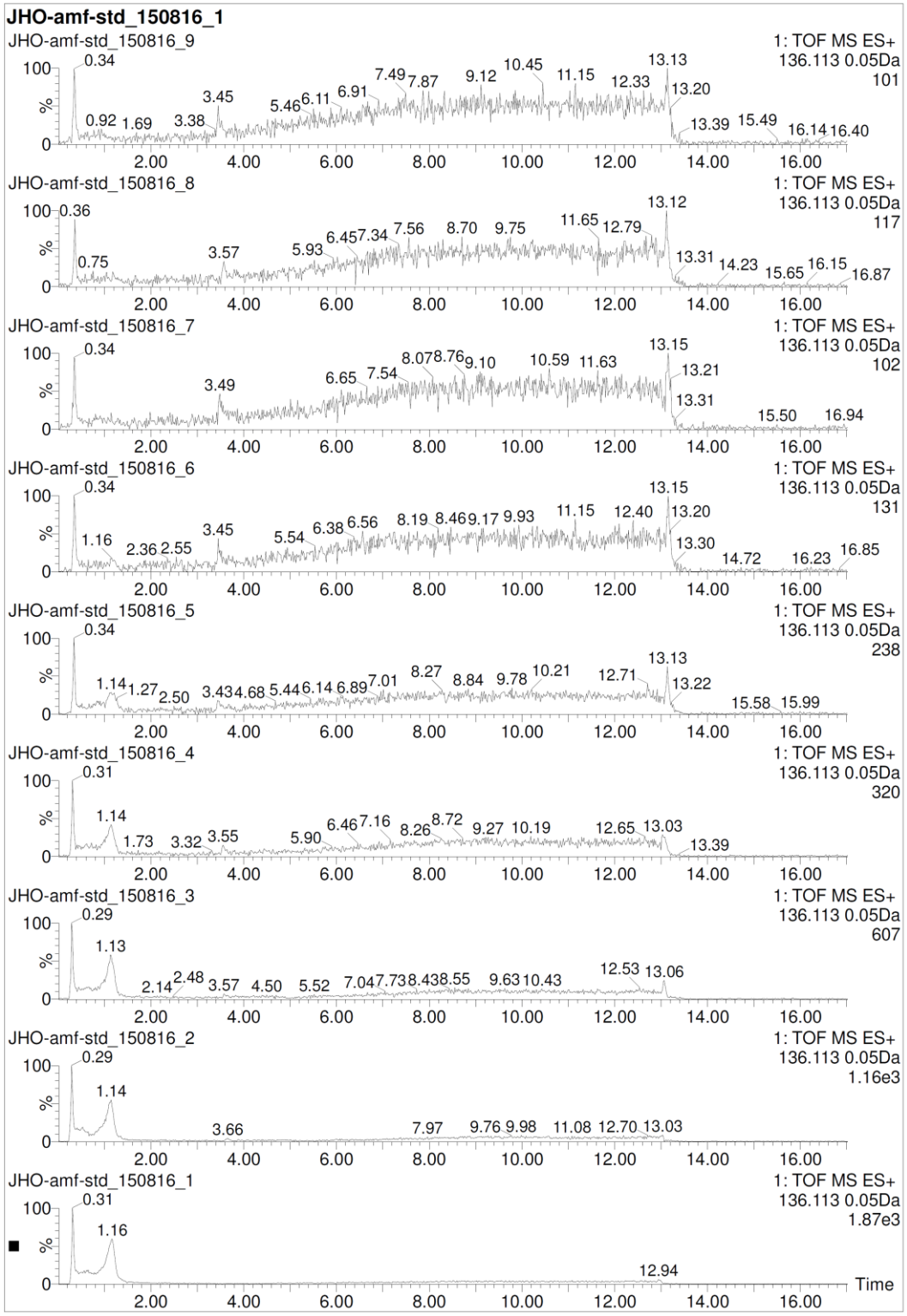


Image 8. TIC chromatogram for a typical experiment with amphetamine standards with concentrations ranging from 100 ng/ml to 0.1 ng/ml.

Table 2. Amphetamine standard series 150816, showing predicted $[M+H]^+$ mass, found $[M+H]^+$ mass, ppm error, normalized i-FIT values and retention time.

Amphetamine standard series 150816	Predicted mass $[M+H]^+$	Found mass $[M+H]^+$	ppm error $[M+H]^+$	i-FIT (normalized)	Retention time (min)
Amphetamine std 1 (100 ng/ml)	136.1126	136.1135	6.6	0.0	1.16
Amphetamine std 2 (50 ng/ml)	136.1126	136.1142	11.8	0.4	1.14
Amphetamine std 3 (25 ng/ml)	136.1126	136.102	-77.9	3.0	1.13
Amphetamine std 4 (10 ng/ml)	136.1126	136.1144	13.2	3.9	1.14
Amphetamine std 5 (5 ng/ml)	136.1126	136.1024	-74.9	1.0	1.14

After a maintenance visit from Waters Inc. the accuracy, technical reliability and reproducibility of the method improved. Samples from amphetamine **1** oils obtained from synthetic routes **I-IV** were prepared in concentrations ranging from 7.86 µg/ml to 17.72 µg/ml in methanol. The goal was that impurities appearing in quantities as low as 0.1% compared with amphetamine **1** (or concentrations as low as 10 ng / ml) would be detected. The experiment-run was designed so that an amphetamine standard (of 0.1 µl/ml) and a blank methanol standard was run before and after the samples from synthetic routes **I-IV**. The TOF-MS was calibrated in the beginning of the day and the same experiment run was completed twice during that day. Table 3 presents the masses from both amphetamine peaks (retention time 0.69 min and 1.69 min) found in the TIC chromatogram of the amphetamine standard, as measured both before and after each of the two runs that day. The ppm error ranged from 5.1 to 14.0 ppm (with mean of 10.1 ppm) and the normalized i-FIT value measured for all peaks was 0.0.

Table 3. Obtained masses from the 0.1 µl/ml amphetamine standard, the ppm error and normalized i-FIT value, as measured before and after each of the two runs on the experiment day.

	Predicted mass $[M+H]^+$	Found mass $[M+H]^+$	ppm error $[M+H]^+$	i-FIT (normalized)	Retention time (min)
Amph. Std. 1st run before (0,1 micro liter / ml)	136.1126	136.1136	7.3	0.0	0.69
amph. Std. 2nd run before (0,1 micro liter / ml)	136.1126	136.1143	12.5	0.0	0.8
Amph. Std. 1st run after (0,1 micro liter / ml)	136.1126	136.1145	14.0	0.0	0.7
Amph. Std. 2nd run after (0,1 micro liter / ml)	136.1126	136.1145	12.5	0.0	0.81
Amph. Std. 1st run before (0,1 micro liter / ml)	136.1126	136.1133	5.1	0.0	1.66
amph. Std. 2nd run before (0,1 micro liter / ml)	136.1126	136.1136	7.3	0.0	1.71
Amph. Std. 1st run after (0,1 micro liter / ml)	136.1126	136.1141	11.0	0.0	1.69
Amph. Std. 2nd run after (0,1 micro liter / ml)	136.1126	136.1141	11.0	0.0	1.71
Average		**136.1140**	**10.1**	**0.0**	

To conduct the non-target screening of impurities from the obtained UPLC-TOF-MS data, a systematic method adopted from Ibáñez, et. al.[71] was employed. The impurities to be screened were selected based on the literature reviewed for Section 4.2.2 in this thesis. Only the results from the first experiment-run of the day were used for the systematic screening, but results were checked from the second run if they appeared unusual in the first run.

First, the MassLynx software was used to build an isotopic model of the screened compounds' $[M+H]^+$ and $[M+Na]^+$ masses. The exact mass predicted by the MassLynx software was screened in the TIC chromatogram obtained from each of the studied samples, using the "find mass"-function. As an example, the chromatograms obtained when searching for the exact mass of the $[M+H]^+$ and $[M+Na]^+$ ion for amphetamine **1** in all the studied samples and the reference samples (before and after the run) are shown in Image 9. Sample JHO-023_050916 corresponds to the amphetamine **1** oil from route **I,** sample JHO-029_050916 corresponds to route **II**, sample JHO-013_050916 corresponds to route **III** and sample JHO-031_050916 to route **IV**.

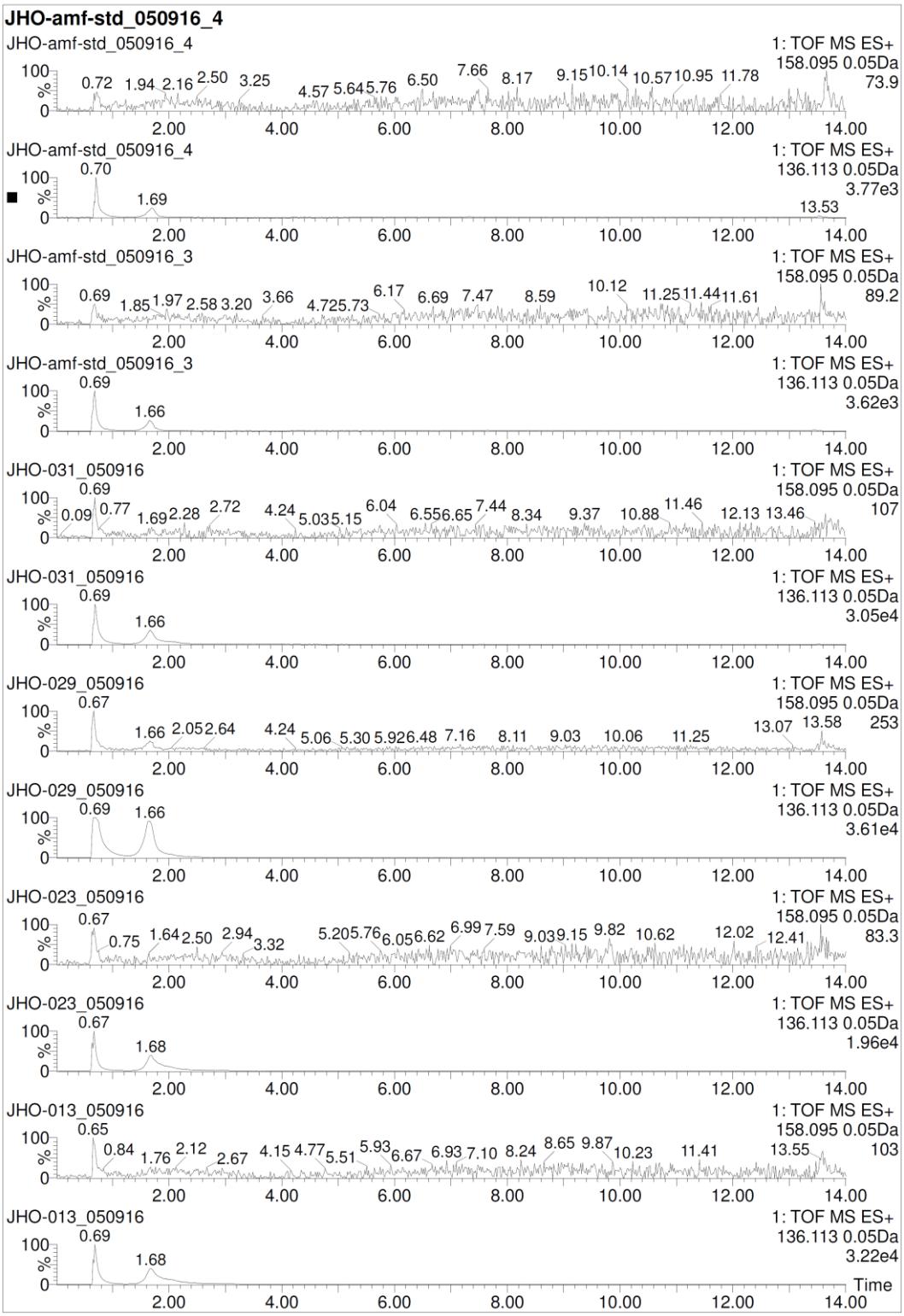


Image 9. The chromatograms for the studied amphetamine samples from synthetic routes **I-IV**, when using the "find mass"-function to search for $[M+H]^+$ and $[M+Na]^+$ ion masses for amphetamine.

Second, the mass spectra of each of the found peaks in the TIC chromatograms (when searching for a specific mass) was obtained by subtracting the mass spectra found on both sides of the peak from the mass spectra found in the middle of the peak. The obtained mass spectra were then compared with the spectra for the predicted isotopic mass distribution of the $[M+H]^+$ and $[M+Na]^+$ ions. As an example, the mass spectra obtained from the peaks with retention times (RT) 0.69 min (from Image 9) are presented in Image 10.

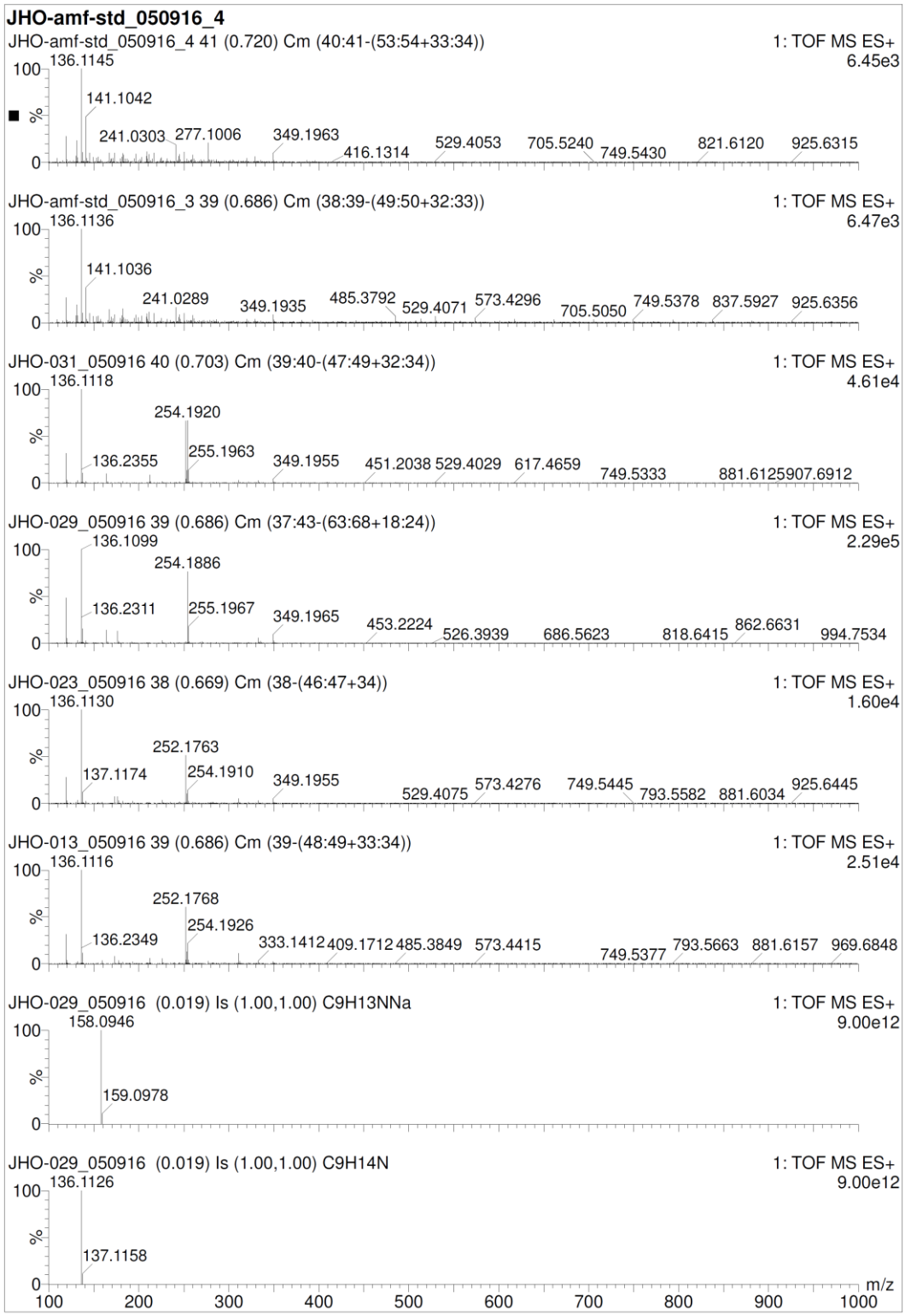


Image 10. The mass spectra gained from the peaks at RT 0.69 minutes in the studied samples (routes **I**-**IV**) and the amphetamine reference (as measured before and after the experiment-run).

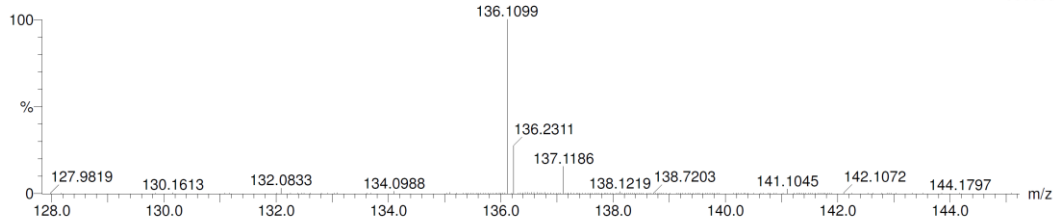
Third, and finally, the elemental composition of interesting peaks matching either the predicted $[M+H]^+$ or $[M+Na]^+$ ion were explored with the "elemental composition"-function. As an example, the elemental composition of mass peak 136.1099 m/z found in route **II** at RT 0.69 min is shown in image 11.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 100.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
146 formula(e) evaluated with 6 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-200 N: 0-200 O: 0-200 Na: 0-1
JHO-029_050916
JHO-029_050916 39 (0.686) Cm (37:43-(63:68+18:24))

1: TOF MS ES+
2.29e+005



Minimum:				-1.5			
Maximum:		5.0	100.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
136.1099	136.1102	-0.3	-2.2	0.5	789.8	2.2	C7 H15 N Na
	136.1086	1.3	9.6	-0.5	792.1	4.6	C4 H14 N3 O2
	136.1126	-2.7	-19.8	3.5	787.7	0.2	C9 H14 N
	136.1059	4.0	29.4	0.5	796.9	9.3	H10 N9
	136.1198	-9.9	-72.7	-0.5	793.1	5.6	C3 H14 N5 O
	136.0974	12.5	91.8	-0.5	791.3	3.7	C5 H14 N O3

Image 11. Elemental composition of mass peak 136.1099 m/z found in route **II** at RT 0.69 min. It is worth noting that the normalized i-FIT value is only 0.2 even if the ppm error is -19.8 compared with the predicted isotopic mass distribution of the $[M+H]^+$ ion for amphetamine.

Because the first part of the two-step biased non-target screening is intended to find, rather than exclude, potentially interesting compounds, and because of the ppm errors found in the mass measurements of the amphetamine reference sample (as shown in Table 3), a 20 ppm error limit was set as the criteria for a positive result. All positive results were recorded in a Table for each studied sample (routes **I-IV**) and the tables can be found in Appendix 5. The found $[M+H]^+$ and $[M+Na]^+$ ions of the screened compounds in the amphetamine **1** oil samples from routes **I-IV** are summarized in Table 4.

Table 4. The found $[M+H]^+$ (designated "H") and $[M+Na]^+$ (designated "Na") ions of the screened compounds in the amphetamine **1** oil samples from synthetic routes **I**-**IV.** An X corresponds to both the $[M+H]^+$ and the $[M+Na]^+$ ion. No prefix indicates the found ion had a less than 5 ppm error to the predicted mass, "<" indicates the error was less than 10 ppm and "<<" indicates an error was less than 20 ppm.

	I benzyl cyanide / leuckart	**II** benzyl cyanide / red. amin.	**III** phenylacetic acid / leuckart	**IV** phenylacetic acid / red. amin.
Amphetamine **1**	H	<< H	< H	H
P2P **5**				
1,3-diphenyl-2-aminopropanone **6**			< Na	Na
1,3-diphenyl-2-aminopropane **3**	< H	H	H	H
Phenylacetic acid **7**				< Na
Benzylphenylethylamineformamide **4**	< X	< X	H	X
APAAN **10**				
Benzyl Cyanide **17**				
4,6-dimethyl-3,5-diphenylpyridine-2-one **9**	<<H & Na	< Na	H	H
12a or **12b**	<< H		< H	
1,5-diphenyl-2-methyl-4-oxopent-1-ene **15**	< Na			
1,5-diphenyl-4-methylpent-1-ene **14**				
Acetylamphetamine **13**	X	X	< X	X
2-oxo-1-phenyl-(beta-phenylisopropylamino)etane **18**	X	< Na	X	X
1-phenyl-2-propanol **16**		< Na		
Acetic anhydride **8**				< Na

When both the $[M+H]^+$ and $[M+Na]^+$ ions are found they should be found with the same retention time if they originate from the same compound. In all the initially screened impurities, except 4,6-dimethyl-3,5-diphenylpyridine-2-one **9**, this was the case. In the case of **9** the $[M+Na]^+$ ions were found with retention times of 4.77-4.84 minutes in routes **I** & **II**, but the $[M+H]^+$ ion seemed to appear in routes **III** & **IV** with retention times 4.28-4.34 minutes (and in route **I** with retention time 4.84 minutes). However, when looking at the alternatives for the elemental composition of mass 276.1377 m/z found in route **III** at

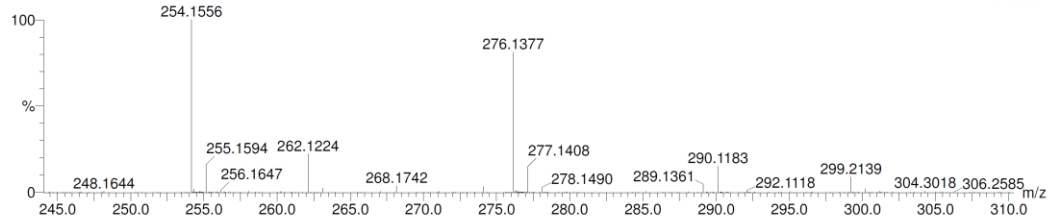
RT 4.34 min, we see that it matches both the $[M+H]^+$ ion of **9** (formula $C_{19}H_{18}NO$) and the $[M+Na]^+$ ion (formula $C_{17}H_{19}NONa$) of a compound with the molecular formula $C_{17}H_{19}NO$, as can be seen from Image 12. Both compounds have a predicted mass within 5 ppm of the found mass and they have i-FIT values of 0.4 and 1.5 respectively.

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 4

Monoisotopic Mass, Even Electron Ions
858 formula(e) evaluated with 7 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-200 N: 0-200 O: 0-200 Na: 0-1
JHO-013_050916
JHO-013_050916 249 (4.379) Cm (247:249-(269:272+230:233))

1: TOF MS ES+
2.07e+004



Minimum:				-1.5			
Maximum:		5.0	10.0	80.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
276.1377	276.1364	1.3	4.7	8.5	179.2	0.4	C17 H19 N O Na
	276.1388	-1.1	-4.0	11.5	180.2	1.5	C19 H18 N O
	276.1369	0.8	2.9	1.5	183.7	4.9	C2 H15 N13 O2 Na
	276.1380	-0.3	-1.1	-0.5	182.8	4.0	C3 H18 N9 O6
	276.1393	-1.6	-5.8	4.5	182.6	3.8	C4 H14 N13 O2
	276.1396	-1.9	-6.9	0.5	181.7	2.9	C6 H19 N7 O4 Na
	276.1367	1.0	3.6	5.5	186.2	7.4	H10 N19

Image 12. Elemental composition analysis of peak 276.1377 m/z found in route **III**, with retention time 4.34 min. The predicted mass for formula $C_{17}H_{19}NONa$ (corresponding to the $[M+Na]^+$ ion of compound **18**) and formula $C_{19}H_{18}NO$ (corresponding to the $[M+H]^+$ ion of compound **9**) are both within 5 ppm of mass 276.1377 m/z.

The other prominent mass peak seen in Image 12 is 254.1556 and it matches the $[M+H]^+$ ion of compound $C_{17}H_{19}NO$ with a less than a 5 ppm error. $C_{17}H_{19}NO$ could be compound 2-Oxo-1-phenyl-(b-phenylisopropylamino)ethane **18**, since it has been previously reported as an impurity found in amphetamine, even if it has not been linked to a particular synthetic route.[6,54]

To summarize, the $[M+H]^+$ ion of compound **18** is only found with retention times 4.28-4.34 minutes and the $[M+Na]^+$ ion of compound **9** is only found with retention times 4.77-4.84 min. It is therefore probable that the peaks around 276.1377 m/z (as seen in image 12) belong to the $[M+Na]^+$ ion of compound **18** when found with a retention time between 4.28 and 4.34 minutes, and to the $[M+H]^+$ ion of compound **9** when found with a retention

time between 4.77 and 4.84 minutes. If we accept this assumption, and we define a positive result as either a $[M+H]^+$ ion or a $[M+Na]^+$ ion found within 20 ppm of the predicted mass, then we can re-interpret the results to form Table 5.

Table 5. Positive results for screened impurities (marked with "X"), when defined as an $[M+H]^+$ or $[M+Na]^+$ ion found within 20 ppm of the predicted exact mass. "G" indicates that the compound has been confirmed with the GC-MS analysis. *See the text for a discussion on compounds **9** and **18**.

	I benzyl cyanide / leuckart	**II** benzyl cyanide / red. amin.	**III** phenylacetic acid / leuckart	**IV** phenylacetic acid / red. amin.
Amphetamine **1**	X G	X G	X G	X G
P2P **5**				
1,3-diphenyl-2-aminopropanone **6**			X	X G
1,3-diphenyl-2-aminopropane **3**	X	X	X	X
Phenylacetic acid **7**				X
Benzylphenylethylamineformamide **4**	X	X	X	X
APAAN **10**				
Benzyl Cyanide **17**				
4,6-dimethyl-3,5-diphenylpyridine-2-one **9***	X	X		
12a or **12b**	X		X	
1,5-diphenyl-2-methyl-4-oxopent-1-ene **15**	X			
1,5-diphenyl-4-methylpent-1-ene **14**				
N-Acetylamphetamine **13**	X G	X G	X G	X G
2-oxo-1-phenyl-(beta-phenylisopropylamino)etane **18***			X	X
1-phenyl-2-propanol **16**		X G		
Acetic anhydride **8**				X

5.4 GC-MS ANALYSIS

The initial plan was to run the harmonized profiling method for amphetamine **1,** presented in Section 4.2.1, on the samples from routes **I**-**IV**, in the forensic laboratory of the National Bureau of Investigation. This would have allowed the synthesized amphetamine **1** oil samples in this study (after crystallization to amphetamine sulfate) to

be compared with the entire database of amphetamine **1** seizure made across Europe. However, after the crystallization attempts there was not enough (between 164 mg and 593 mg, depending on the sample) crude amphetamine **1** base for the full profiling method. Consequently, the synthesized samples from routes **I**-**IV** were analyzed with the routine GC-MS method employed by the NBI Forensic Laboratory for identification of drugs and their impurities, using their own and Wiley's compound libraries. The method is accredited by the Finnish Accreditation Service (FINAS). Also the crystallized amphetamine sulfate from routes **II** & **IV** were analyzed with this method.

The full results from the GC-MS analysis are presented in appendix 8. The first thing to note is the high purity of the crystallized amphetamine sulfate samples compared with the oil samples. The amphetamine sulfate sample from route **II** contained only small amounts of di-(-phelisopropul)amine (CAS 99833-41-2) and 1,3-dimethyl-2-phenyl-naphthalene (CAS 998273-40-9) in addition to amphetamine **1.** The amphetamine sulfate sample from route **IV** contained only a small amount of benzylphenethylamine (CAS 998213-06-1) in addition to amphetamine **1.**

In the oil samples from routes **I**-**IV** 11 to 18 different impurities was identified (in addition to amphetamine **1** and the internal standards). Of the compounds screened with the UPLC-TOF-MS method the presence of amphetamine **1** and *N*-Acetylamphetamine **13** in routes **I**-**IV**, 1-phenyl-2-propanol **16** in route **II** and 1,3-diphenyl-2-aminopropanone **6** in route **IV** was confirmed. The compounds confirmed with the GC-MS method are marked with a "G" in Table 5.

6 DISCUSSION AND FURTHER RESEARCH

The present study employed a multidisciplinary approach to chemical impurity profiling of illicitly produced amphetamine **1**. Based on the literature on the illicit amphetamine **1** market in Finland and Europe, the globally seized amphetamine pre- and pre-precursors, and the most commonly used illicit manufacturing methods, the synthetic routes to be studied in this research were determined. The role of chemical impurity profiling of drugs in intelligence-led policing strategies was explored, and the particular requirements of analytical methods employed in service of the forensic intelligence process were identified. Using this background knowledge, permission to conduct research on controlled substances was applied and received from FIMEA.

6.1 IDENTIFIED ROUTE-SPECIFIC IMPURITIES

Amphetamine was synthesized by four distinct routes **I**-**IV** (Scheme 2), using two different pre-precursors (benzyl cyanide **17** and APAAN **10**) and two different methods (the Leuckart reaction and reductive amination) to convert P2P **5** to amphetamine **1.** The synthesized amphetamine **1** samples were analyzed with UPLC-TOF-MS, using a biased non-target screening method, and GC-MS, using mass spectral libraries.

Several potential route-specific impurities were tentatively identified by the UPLC-TOF-MS method, as shown in Table 5. 4,6-dimethyl-3,5-diphenylpyridine-2-one **9** was only identified in the two amphetamine **1** samples made from benzyl cyanide **17** (routes **I** & **II**), in accordance with Power et. al.'s earlier finding.[10] This confirmed the second hypothesis set in the beginning of this study. However, *alpha*-benzylphenethylamineformamide **4** was found in all routes **I**-**IV**, contrary to previous reports[6,59] and contrary to the first hypothesis set in this study.

Molecular formula $C_{17}H_{19}NO$ (suspected of being compound **18**[54]**)** and 1,3-diphenyl-2-aminopropanone **6** was identified only in the two amphetamine **1** samples made from phenylacetic acid **7** (routes **III** & **IV**). Compound **18** has not previously been linked to a particular synthetic route.[6,54] The presence of **6** was confirmed by GC-MS only in the amphetamine **1** sample made from **7**, when the Leuckart reaction was used for the final step (route **IV**). The presence of **6** was confirmed by NMR in all P2P **5** samples made from **7** (but not in the two P2P **5** samples made from benzyl cyanide **17**), in accordance with previous reports.[6,35] 1,3-diphenyl-2-aminopropane **3** and

Benzylphenylethylamineformamide **4**, which previously have been reported to form *only* when P2P **5** contaminated with 1,3-diphenyl-2-aminopropanone **6** is used[6,59,60], were now identified also in amphetamine **1** made from P2P **5** *not* containing **6** (in routes **I** & **II,** which start from benzyl cyanide **17**).

Molecular formula $C_{19}H_{17}N$, belonging either to 2,6-dimethyl-3,5-diphenylpyridine **12b** or 2,4-dimethyl-3,5-diphenylpyridine **12a** was identified only in the two routes **I** & **III** using the Leuckart reaction as the last step, in accordance with previous reports.[6,63]

1-phenyl-2-propanol **16** was identified both by the UPLC-TOF-MS method and GC-MS analysis in reductive amination route **II** (starting from benzyl cyanide **17**), but not in reductive amination route **IV** (starting from phenylacetic acid **7**). **16** has previously been identified as a route-specific impurity for methylamphetamine **2** made by reductive amination, using the same Al/$HgCl_2$ catalyst as in this study.[58] It has also been indicated that if an excess of ammonia is *not* used in the reductive amination of amphetamine **1** 1-phenyl-2-propanol **16** could form as a side reaction[6,31]. However, in this study 400 mol-% of ammonia was used both in route **II** & **IV.** It is worth noting that the P2P **5** in route **IV** contained 1,3-diphenyl-2-aminopropanone **6** as a minor product in a roughly 30:70 ratio, but the P2P **5** in route **II** did not. To clarify why **16** was only found in route **II**, but not in route **IV**, its formation could first be isolated to the last step, by screening for it in the P2P **5** used in all routes **I**-**IV**. Then the influence of the amount of ammonia on the formation of **16** could easily be established with a series of reductions using different amounts of ammonia. Finally, the influence of 1,3-diphenyl-2-aminopropanone **6** on the formation of 1-phenyl-2-propanol **16** could be explored by reducing clean **5** and **6** in mixtures of different rations.

Finally, it is worth noting that even if the 1-phenyl-2-propanol **16** in route **II** was found from the amphetamine **1** crude oil, it was not found from the amphetamine sulfate crystallized from it. The previously mentioned study on methylamphetamine **2** found **16** from an acidic, but not from a basic extract of the studied methyamphetamine **2** chloride.[58] The used GC-MS method in this study used only a basic extract, which could explain this finding. However, another reason could be that the concentration of **16** in the purified amphetamine sulfate crystals simply is too low for the sensitivity of the employed GC-MS method.

6.2 Reliability of the UPLC-TOF-MS Method

Based on the performance of the UPLC-TOF-MS method, when analyzing the amphetamine **1** reference standards (shown in Table 3), a less than 20 ppm error seemed attainable and was used as criteria for a positive identification of $[M+H]^+$ or $[M+H]^+$ ions. The mean error was 10.1 ppm, which means +-0.0014 m/z for amphetamine **1**. This is consistent with the accuracy obtained by Nielsen. el. al., on which the UPLC-TOF-MS method used in this study is based. Nielsen et. al. reported results within +-6.72 mDa (~0.0067 m/z, in a single charged state) for compounds in the 100-400 m/z range. The mean error was +-1.82 mDa (~0.0018 m/z, in a single charged state).[70]

The reliability of HRMS analysis depends on a sufficient resolution to separate isotopic masses from each other and a sufficiently small accuracy error to narrow down the list of possible molecular formulas. The calibration of the used TOF-MS method in the beginning of each day always gave a resolution of >10000, which is enough to give separate peaks for different isotopic mass compositions.[75,76] The number of possible molecular formulas within a given ppm error depends on the size of the molecule (fewer atoms means fewer possible permutations), as illustrated in Image 13, but also on what other information is known about the molecule.[76,77] For example, a 118 m/z compound with $C_{0\text{-}100}H_{3\text{-}74}O_{0\text{-}4}N_{0\text{-}4}$ only needs a 34 ppm accuracy to be unambiguously identified. However, the corresponding accuracy for a 750 m/z compound with $C_{0\text{-}100}H_{25\text{-}110}O_{0\text{-}15}N_{0\text{-}15}$ is 0.018 ppm (unachievable with any equipment today) to eliminate all alternative formulas.[78]

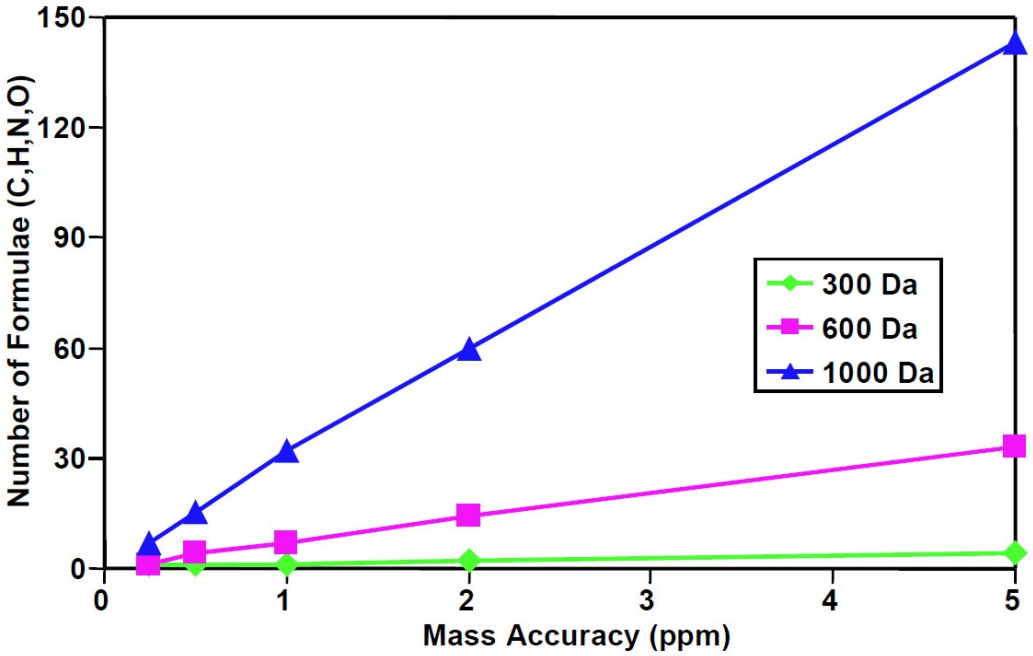


Image 13. The influence of the accuracy (in ppm) on the number of possible molecular formulas for different sized molecules.[76]

The Journal of Organic Chemistry (JOC) – Guidelines for Authors (updated January, 2016) suggest a +-0.003 m/z accuracy for HRMS identification, when used together with other available data (like what elements the potential molecule is composed off).[79] This translates into a 5 ppm error for 600 m/z compounds, 10 ppm error for 300 m/z compounds and a 20 ppm error for 150 m/z compounds. Looking back at Table 4, we see that all other positive results, except the $[M+H]^+$ ion for compound **9** in route **I** fall within the JOC suggestion (the found $[M+H]^+$ ion for compound **12a** or **12b** in route **I** and the $[M+H]^+$ ion for amphetamine **1** in route **II** are within 0.003 m/z of the target masses). However, as the presence of compound **9** in route **I** is suggested also based on the $[M+Na]^+$ ion, there is no change in the analyzed results presented in Table 5 if we use the criteria suggested by JOC. In other words, the 20 ppm accuracy set as the criteria for positive results seems reasonable for the molecular weight-range that the studied compounds fall into.

However, the accuracy of the TOF-MS measurements could be better. With a method optimized for the analyzed compounds a smaller than 5 ppm error can be achieved with TOF-MS instruments (especially when analyzing pure compounds of known quantities). However, in practice that is not always the case, as illustrated in the best practice guide

from the National Measurement System in the UK.[76] In it, a presented inter-laboratory study found that 85% of quadrupole-TOF measurements were within 5 ppm of the target mass, but the corresponding figure for conventional TOF measurements was only 65%. Most orthogonal-TOF instruments showed smaller than 10 ppm errors. The same guide notes that, "The key parameter to consider when using a lock mass with TOF and quadrupole-TOF instruments is the relative and absolute abundance of the analyte and lock (reference) ions." This may be one explanation for the larger than 5 ppm error observed in some of the results in this study. The Leucine Enkhephaline (LeuEnk) standard used as lock-mass had a concentration of 1000 ng/ml and the analyte concentrations could be anywhere between 10 000 ng/ml and 10 ng/ml (close to the limit of detection, as seen in Image 8 and Table 2). Better results could most likely be obtained if the concentrations for the analytes and the used LeuEnk standard were closer to each other.

Another way to reduce the number of possible molecular formulas within a certain ppm error range is to use normalized i-FIT values as well. Waters Inc. has developed an algorithm to predict the number of carbon, sulfur and chloride or bromide atoms based on the relative intensity of the 1st and 2nd isotope peaks.[80] Using these predicted atomic compositions (+-3 for carbon atoms) the number of possible molecular formulas within the given ppm range was reduced significantly. In other words, using a suitable ppm error combined with the normalized i-FIT value (and other information about the molecule, for example the rough number of carbon atoms) to limit the number of possible molecular formulas gives better results than using only one of them alone.

6.3 Using the Results in the Forensic Intelligence Process

The UPLC-TOF-MS results in this study should be considered as the first step in the two-step biased non-target screening employed by Ibáñez, et. al.[71] The next step would be to synthesize or purchase reference standards for the most interesting impurities to confirm their existence using the retention time, exact mass and isotopic distribution. This would be the easiest and quickest way to confirm the UPLC-TOF-MS results presented in Table 5. As discussed in Section 4.3, there is no need to re-analyze the samples synthesized for this study, only re-treatment of the data is needed. If a validated UPLC-TOF-MS method would routinely be used in forensic laboratories and the data would be stored in an easily accessible database, new impurities could be identified in old samples in a similar manner.

However, as also discussed in Section 4.2, "Systems implementing the forensic intelligence process must be pragmatic enough to sustain uncertain reasoning while remaining scientifically rigorous and controllable."[81] That is why it has been suggested that forensic analysis in general should move towards a two-step approach: a rough and rapid analysis for detection, followed by a longer, more rigorous analysis for confirmation.[47,81,82] The similarity to the two-step approach employed in this study is apparent. Thus, as a source of forensic intelligence, the results from this study (even without confirmation using references) could be useful, especially if combined with established impurity profiling methods and other forms of intelligence (like interrogations of a suspect seized in relation to a raid of an illicit amphetamine **1** laboratory).

Once the route-specific impurities tentatively identified in this (or any other) study have been confirmed, those compounds *alone* could be used to create a theoretical route or precursor specific class, akin to Image 4.[39] The impurity profile of any new seizure could then be compared to it, using *only* the route-specific impurities. Similar statistical methods to the ones already used to link profiles of seized drug samples to each other could be utilized.[54] Now the remaining, *not* route-specific impurities could be used to answer the questions posed by Morelato et. al. about the inhomogenous methylamphetamine **2** samples; were the seizures made by different people in different laboratories, or was it possibly the same person and laboratory using different methods and starting materials?[56] In other words, by identifying the impurities that vary based on the synthetic route or used starting material, we could use the variation in the *other* impurities to link seized samples to a particular chemist or laboratory. Alternatively, if all impurities are used, they could be weighted differently in the statistical analysis, depending what kind of link is sought.

6.4 The Need For and Challenges of Further Research

As concluded in Chapter 2, illicit amphetamine **1** manufacturing (and illicit synthetic drug manufacturing in general) is getting more professional, and new synthetic routes and starting materials are constantly adopted to circumvent legislation. This makes it challenging for forensic laboratories to keep up, especially when harmonized development of new methodologies can take several years.[48] One answer to this problem could be the adoption of multi-stage analytical methods, where new compounds could be identified in old samples, simply by obtaining new references, as discussed earlier.

Another solution could be an increased co-operation between forensic laboratories and universities.[43] However, as demonstrated in Section 5.1, the permit application process needed to conduct research on controlled substances is long and the support offered by FIMEA is insufficient, to the point where doing academic research on controlled substances might become too tedious to be feasible in the long run. In this sense, the worry expressed in the INCB special report, "Availability of Internationally Controlled Drugs: Ensuring Adequate Access for Medicinal and Scientific Purpose" seems warranted: "Access to internationally controlled substances might also be unduly restricted out of fear of their diversion into illicit channels, as well as fear of prosecution or sanction."[11] If FIMEA (and other regulatory bodies) were to offer better support, guidelines and best practices on how the necessary permit applications should be made, they could reduce the barrier to conduct legitimate academic research on controlled substances, make it worthwhile for academics in the long run and for their part promote further collaboration between forensic laboratories and universities.

Finally, as demonstrated throughout this study, forensic intelligence is a highly multi-disciplinary field. However, "[t]he over-specialization [of forensic science disciplines] and the gap between forensic science [as practiced in forensic laboratories] and policing can [also] be considered as obstacles [for creating holistic forensic intelligence frameworks] …".[81] That is why we need a simultaneous understanding of the relevant questions (in intelligence-led policing), the tools available to answer them (like chemical profiling methods) and the process by which the provided answers are most effectively utilized. Academic research in universities seems ideally suited for facilitating this synthesis of disciplines.

7 Experimental Procedures

7.1 Synthetic Procedures

7.1.1 Preparation of Amphetamine 1 from P2P 5 via The Leuckart Reaction[36]

Crude P2P **5** oil (1.66 g, 100 m-%) was mixed with formamide (531 mg, 32 m-%) and formic acid (266 mg, 16 m-%) in a round-bottom flask. The mixture was heated with stirring at 150 °C for 5h, after which it was allowed to cool to room temperature and HCL (32%, 8,8 ml) was added. The mixture was then refluxed at 110 °C for 2h, after which it was allowed to cool to room temperature, diluted with H_2O, made basic with a NaOH-solution, extracted with DCM and dried over $MgSO_4$. Solvents were evaporated to give 1.437 g of brown crude amphetamine **1** oil.

^{1}H and ^{13}C NMR ($CDCl_3$) was run on the crude product to find characteristic peaks and tentatively identify the main components.

7.1.2 Preparation of Amphetamine 1 from P2P 5 via Reductive Al/$HgCl_2$ Amination[74]

Aluminium foil (1.8 g), EtOH (108 ml) and $HgCl_2$ (332 mg, 1.2 mmol, 1.2 mol-%) was put in round-bottom flask and stirred at 0 °C for 30 min. Crude P2P **5** oil (1.8 g, 13.4 mmol, 100 mol-%) and NH_3 (2 mol/l, 54 ml, 54 mmol, 400 mol-%) was added into a separate flask at 0 °C. The P2P **5** / NH_3 solution was added slowly to the aluminium foil / $HgCl_2$ solution at 0° C. The mixture was heated to 72 °C, held for 2h with stirring and cooled to room temperature, after which it was diluted with a saturated KOH/H_2O-solution. The remaining solid aluminium was filtered, the solution was extracted with CH_3Cl_2, dried with $MgSO_4$ and concentrated in vacuum to give 1.121 g of yellow crude amphetamine **1** oil.

The amphetamine **1** oil was crystallized to amphetamine sulfate, by mixing the oil with acetone in a 1:3 ration, adding a few drops of ~30% H_2SO_4 and letting the mixture stand in an ice bath for 1h. The formed crystals were filtered, washed with cold acetone and dried in a 70 °C oven for 4h.

The amphetamine **1** oil was crystallized to amphetamine chloride, by mixing the oil with acetone in a 1:3 ration, adding a 0.1 ml 32% HCl and letting the mixture stand in an ice bath for 2h. The formed crystals were filtered, washed with cold acetone and dried in a 70 °C oven for 4h.

^{1}H and ^{13}C NMR ($CDCl_3$) was run on the crude product to find characteristic peaks and tentatively identify the main components.

7.1.3 Preparation of P2P 5 from Phenylacetic Acid 7[35]

A round-bottom flask was flame dried and put under argon atmosphere. Phenylacetic acid **7** (6.81 g, 50 mmol, 100 mol-%) was put in the flask, dissolved in acetic anhydride **8** (25 ml, 250 mmol, 500 mol-%) and purged with argon gas at room temperature for 15 min. Pyridine (1.86 ml, 25 mmol, 50 mol-%) was added and the mixture was heated to 150 °C under stirring. After 20h the reaction was quenched with H_2O, allowed to cool to room temperature, extracted with ethyl acetate (3 x 50 ml), washed with $NaHCO_3$ (2 x 50 ml) and dried with $MgSO_4$. After concentration in vacuum, 7.473 g of yellow crude P2P **5** oil was obtained**.** The crude P2P **5** oil was purified once with flash chromatography (20% EtOAc/Hexane) to give a reference compound, which was used to confirm the presence of P2P **5** in the crude oils used in the research reactions.

^{1}H-NMR ($CDCl_3$): δ = 2.15 (s, 3H), 3.70 (s, 2H), 7.20-7.36 (m, 5H)

^{13}C-NMR ($CDCl_3$): δ = 29.4, 51.1, 127.2, 128.9, 129.5, 134.4, 206.5

7.1.4 Preparation of P2P 5 from APAAN 10[36]

APAAN **10** (6.34 g, 39.8 mmol) was mixed with H_2O (38 ml) and cooled to 0 °C in a round-bottom flask. Conc. H_2SO_4 was added slowly, after which the temperature was risen to 150 °C under vigorous stirring. After 2h the mixture was cooled to room temperature, and the solids were filtered and dissolved in H_2O. The pH was adjusted to 5 with conc. HCl and the solution was extracted with EtOAc, dried with $MgSO_4$ and concentrated in vacuum to give 2.111 g of a crude P2P **5** yellow paste.

^{1}H and ^{13}C NMR ($CDCl_3$) was run on the crude product and compared with the purified P2P **5** NMR spectra to confirm the existence of P2P **5** in the crude product.

7.1.5 Preparation of APAAN 10 from Benzyl Cyanide 17[73]

Benzyl cyanide **17** (10.05 ml, 87.07 mmol, 100 mol-%) was dissolved in EtOAc (200ml) in a round-bottom flask. Metallic natrium (2.8 g, 121.795 mmol, 140 mol-%), stored in oil, was cut, washed in isopropanol and added to the mixture. The mixture was heated to 80 °C and held for 18 h under stirring. The mixture was allowed to cool to room temperature,

the solids were collected by filtration and dissolved in H_2O. The solution was made acidic with conc. HCl, extracted with EtOAc (2 x 20 ml), dried with $MgSO_4$ and concentrated in vacuum to give 6.339 g of a light yellow crude APAAN **10** powder.

^{1}H and ^{13}C NMR ($CDCl_3$) was run on the crude product to find characteristic peaks and tentatively identify the main components.

7.2 Analytical Parameters

7.2.1 UPLC-TOF-MS[70]

Samples were prepared by dissolving between 11.8 and 26.6 mg of sample into 3 ml of methanol. The samples were then diluted to 7.86-17.72 µg/ml in 50 ml volumetric flasks. 0.8 ml of sample was transferred into vials and put into the autosampler. From a 1 µg/ml amphetamine reference received from the NBI Forensic Laboratory a 100 ng/ml sample was prepared and used as reference.

Chromatographic separation was performed on a 1.0 mm x 50 mm Aquity BEH C18 1.7 µm column, using an Aquity Ultra-Performance Liquid Chromatography System from Waters Inc. The mobile phase was composed of A (0.05% formic acid in H_2O) and B (100% methanol). The column was maintained at 35 °C and eluted with a gradient of 10% B (0-0.5 min), 10-50% B (0.5-4.0 min), 50-100% B (4.0 – 11.0 min), flushed at 100% B (11.0-13.0 min) and dropped back to 10% B (13.0-14.0 min). The flow rate was 0.3 ml/min and the autosampler was maintained at 12 °C.

The mass spectrometry was performed on a Micromass LCT Premier orthogonal acceleration Time-of-Flight mass spectrometer from Waters Inc., operated in positive ion mode with electrospray ionization. The nebulization gas was set to 750 L/h at a temperature of 350 °C. The cone gas was set to 10 L/h and the source temperature was set to 120 °C. The capillary voltage and the cone voltage were set to 3000 and 30 V, respectively. The aperture 1 voltage was set to 10 V. The LCT Premier was operated in W optics mode with a >10000 resolution after calibration. Scan time was set to 1 s. Leucine-enkephaline was used as lock mass at a concentration of 1000 ng/ml and a flow rate of 2 µl/min. The lock mass automatically sampled every 5 scans. Data was collected in centroid mode from *m/z* 100-1000. The results were analyzed using the MassLynx software.

7.2.2 GC-MS

The samples were prepared by dissolving 5 – 20 mg of sample into a 1 ml solution of L-valine ethyl ester (0.5 mg/ml). Then the samples were extracted with 1ml of a butyl acetate solution containing α-cholestane (0.1 mg/ml) and centrifuged. Filtration was performed if needed using 17 mm Whatman filters. Approximately 0.8 ml of the butyl acetate layer was transferred into a 2 ml vial and placed into the autosampler for analysis.

The samples were analyzed with an Agilent GC 7890A gas chromatograph, coupled to an Agilent MS 5973C mass spectrometer. An Agilent DB-5, 10 m x 0.1 mm, d_f 0.10 µm column was used in split mode (93:1 ratio), using a helium carrier gas at constant flow 40 ml/min. The injector was set at 250 °C and the transfer line at 350 °C. The initial oven temperature was 90 °C and it was increased with 45 °C / min to a final temperature of 320 °C with a final hold time of 0.5 min. *α*-kolestaan was used to RTloc with a 0,4 ml/min flow. The mass spectrometer was set in scan mode in *m/z* range 35-500, with an ionization energy of 70 eV. L-valine ethyl ester 0.5 mg/ml and α-cholestane 0.1 mg/ml was used as internal standards. The samples were identified using internal and commercial (from Wiley's) mass spectral libraries.

7.2.3 ^{1}H AND ^{13}C NMR

The NMR spectra were recorded with a Bruker Advance DPX-400 spectrometer (^{1}H 399.98 MHz, ^{13}C 100.59 MHz). The chemical shifts are reported in ppm relative to the residual solvent signal (^{1}H: $CDCl_3$ 7.26, ^{13}C: 77.16). The results were analyzed using the TopSpin 3.1 software.

7.2.4 GENERAL

Reagents were obtained from TCI and Sigma Aldrich. Reactions were monitored the first time with TLC analysis (Merck silica gel 60 F_{254}, 230-400 mesh, aluminium) and the plates were visualized with UV (254 nm)) and standard solutions of phosphomolybdic acid and permanganate.

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APPENDICES

Appendix 1: Censored handling permit application for Amphetamine (in Finnish)

Appendix 2: Censored manufacturing permit application for Amphetamine (in Finnish)

Appendix 3: The answer to the request for addition information from FIMEA (in Finnish)

Appendix 4: Research plan from 22nd of February 2016 (in Finnish)

Appendix 5: The results from the UPLC-TOF-MS analysis

Appendix 6: The spectra from the UPLC-TOF-MS analysis

Appendix 7: ^{1}H and ^{13}C NMR spectra for the synthesized substances

Appendix 8: The results from the GC-MS analysis

Appendix 9: GC-MS chromatograms

Appendix 1: Censored handling permit application for Amphetamine in Finnish

Huumausaineiden käsittelylupahakemus (373/2008 15 §)

1 Organisaation tiedot

Aalto-yliopisto
Kemian tekniikan korkeakoulu
Y-Tunnus: [redacted]
Organisaatio kaavio Liitteessä 1

Postiosoite
Aalto-yliopisto
Kemian tekniikan korkeakoulu
PL 16100
00076 AALTO

Käyntiosoite
[redacted]

Kemian tekniikan korkeakoulu
Kemian Laitos
Organisaatiokaavio Liitteessä 2

Postiosoite
PL 16100
00076 AALTO

Käyntiosoite
[redacted]

2 Tiedot luvanhakijan soveltuvuudesta (373/2008 11 §)

[redacted]
Kemiantekniikan korkeakoulun dekaani (kts. Organisaatiokaavio Liite 1).
Kirjallinen vakuutus, Liite 3.

3 Toiminnan kuvaus

3.1 Huumausaineluvan alaisten toimintojen laatu ja laajuus

Kemian laitoksen opetus ja tutkimus käsittelee kaikkia kemian pääsuuntia, jotka ovat epäorgaaninen, orgaaninen, analyyttinen, fysikaalinen ja laskennallinen kemia. Laitos tekee korkeatasoista perus- ja soveltavaa tutkimusta sekä kouluttaa päteviä ja haluttuja kemian osaajia elinkeinoelämän ja yhteiskunnan palvelukseen.

Huumausaineluvan alaista tutkimusta tehdään [redacted].
Tutkimuksessa joudutaan käsittelemään ja syntetisoimaan luvanvaraista huumausainetta ja niiden prekursoreita, väli-, sivu- tai lopputuotteena, huumausaineita muistuttavien aineiden tutkimuksessa.

Amfetamiinin valmistus on välttämätöntä kyseisen aineen synteettisen epäpuhtausprofiilin tutkimiseen. Tutkimus liittyy ”Luonnonainetutkimus”-nimiseen projektiin. Tutkimuksen kohteena on eri synteesireittejä pitkin syntyvät karakteristiset epäpuhtaudet, joiden avulla voidaan päätellä huumausaineen alkuperä. Tämän vuoksi kyseistä ainetta on valmistettava eri synteesireittejä pitkin, sellaisia määriä, että myös kiinnostavat epäpuhtaudet, joita voidaan olettaa syntyvän noin 1-5% synteesin päätuotteesta voidaan eristää ja karakterisoida. Yhden synteesin aikana tehdään enintään [redacted] amfetamiinia ja sitä säilötään vain niin kauan, että se saadaan analysoitua. Tämän vuoksi varastoitua amfetamiinia on kerralla enintään [redacted] Tehtävät synteesit ovat eritelty liitteessä 4.

Tutkimusta varten joudutaan käsittelemään yhteensä ja enintään seuraavaa määrää luvanvaraista ainetta:

Amfetamiini: [redacted] (373/2008 3§ momentti 5b)

3.2 Huumausaineen tilaus ja vastaanotto, sekä varastointi ja luovutus

3.2.1 Huumausaineiden tilaus ja vastaanotto

Huumausaineita saa tilata ainoastaan kohdassa 5, 6 ja 7 nimetyt vastuu- ja varavastuuhenkilöt. Huumausaineet tilataan etabloiduilta kemikaalitoimittajilta, joilla on lupa myydä ja toimittaa niitä. Huumausaineiden toimituksessa lähetyksen saa kuitata vastaanotetuksi ainoastaan kohdassa 5, 6 ja 7 nimetyt vastuu- ja varavastuuhenkilöt. Käytännössä, toimituksen saapuessa vahtimestari on yhteydessä vastuu- tai varavastuuhenkilöön, joka tulee henkilökohtaisesti vastaanottamaan toimituksen. Vastaanoton jälkeen lähetys siirretään kohdassa 3.2.2 kuvattuun varastoon.

3.2.2 Huumausaineiden varastointi

Kemian laitoksella on huumausaineille yhteinen kaappi, jossa säilötään kaikki luvanvaraiset huumausaineet. Kaappi on [redacted] Avaimia on [redacted] kpl ja niiden hallinnasta päättä kohdassa 5 määrätty vastuuhenkilö. Tällä hetkellä avaimet ovat seuraavien henkilöiden hallussa:

Nimi Sotu. Rooli organisaatiossa

Kaappi sijaitsee [redacted], metallioven takana, joka on lukittu sähköisellä lukolla. Huoneeseen on pääsy [redacted] sähköisellä kulkuluvalla. Kulkukortin käytöstä jää loki. Kulkukorttien luovutuksessa noudatetaan Kemian tekniikan korkeakoulun yleisiä ohjeita.

3.2.3 Huumausaineiden luovuttaminen varastosta ja kirjaaminen varastoon

Huumausaineita luovutetaan varastosta ja kirjataan varastoiduksi yhtenäisellä käytännöllä. Huumausaineiden luovutuksen ja varastoinnin yhteydessä kaksi henkilöä, joista toinen on kohdassa 5, 6 ja 7 määrätty vastuu- tai varavastuuhenkilö, punnitsee ja kirjaa kohdan 3.4 mukaisen kirjanpitokäytännön mukaisesti luovutetun tai varastoidun huumausainemäärän kirjanpitoon. Vaaka ja huumausainekirjanpito sijaitsevat [redacted]

Huumausaineita saa luovuttaa vain kohdassa 5, 6 ja 7 esitetyt vastuu- ja varavastuuhenkilöt tilapäisesti luotettavalle tutkijalle tutkimuskäyttöön. Tutkija on tällöin henkilökohtaisesti vastuussa hänelle luovutetusta huumausaineesta.

3.2.4 Huumausaineiden kuljettaminen

Huumausaineita ei kuljeteta toimipisteestä toiseen.

3.3 Huumausaineiden hävityskäytännöt

Huumausaineet hävitetään liuottamalla ne sopivaan liuottimeen ja siirtämällä ne [redacted] liuotinjäteastiaan. Liuotinjäteastiat toimitetaan Ekokemille käsiteltäväksi Kemian laitoksen yhteisten jätteenkäsittelykäytäntöjen mukaisesti. Huumausaineiden erottelu liuotinjäteastiasta on erittäin hankalaa ja mahdotonta ilman tarkoituksen mukaista laboratoriolaitteistoa.

3.4 Huumausainekirjanpidon kuvaus

Huumausaineiden luovuttamisesta varastosta, tutkimuskäytöstä ja varastoinnista pidetään kirjaa. Kirjanpitoon merkitään seuraavat asiat: huumausaineen nimi, hankinta tai tekopaikka, otettu tai varastoitu määrä, käyttötarkoitus, jäljellä oleva määrä, kahden henkilön nimikirjoitus, joista toinen on kohdassa 5, 6 tai 7 nimetty vastuu- tai varavastuuhenkilö, sekä päivämäärä.

Kirjanpito tehdään tarkoituksenmukaiseen laboratoriopäiväkirjaan kuulakärkikynällä.

Kerran vuodessa tehdään FIMEA:n ohjeiden mukainen inventaario varastossa olevista huumausaineista ja FIMEA:lle toimitetaan kuluneen vuoden huumausainekirjanpito.

4 Selvitys toimitiloista

4.1 Kemian laitos

Käyntiosoite
[redacted]

[redacted]

4.2 Kemian laitoksen vastuuhenkilö

Kemian laitoksen vastuuhenkilö on [redacted] (lisätiedot kohdassa 5).

4.3 Kuvaus varastoinnista, kulkuluvista ja henkilökunnan määrästä

Kuvaus varastointihuoneesta ja sen kulkuluvista on annettu kohdassa 3.2.2. Kemian laitoksen toimipaikassa työskentelee yhteensä [redacted] henkilöä.

4.3.1 Kuvaus varastointihuoneesta, sen kulkuluvista ja henkilökunnan määrästä

Kuvaus varastointihuoneesta ja sen kulkuluvista on annettu kohdassa 3.2.2. Varastointihuoneeseen on pääsy [redacted] henkilöllä.

4.3.2 Kuvaus työskentelytiloista, niiden kulkuluvista ja henkilökunnan määrästä

Kohdassa 3.2.3 mainitut luotettavat tutkijat työskentelevät yhdessä laboratoriossa, jonka huonenumero on [redacted]. Työskentelyn aikana huumausaineet ovat koko ajan luotettavan tutkijan valvonnan alaisina. Laboratorio pidetään koko ajan lukittuna ja sinne pääsee vain [redacted]. Laboratorioon on pääsy [redacted] henkilöllä.

Lisäksi edellä mainitut tutkijat tekevät lyhytkestoisia analyysejä huoneissa [redacted] ja [redacted].

5 Tiedot vastuuhenkilöstä (373/2008 16 §):

5.1 Vastuuhenkilön nimi ja henkilötunnus:

[redacted] Sotu: [redacted]

5.2 Vastuuhenkilön yhteystiedot:

[redacted]

5.3 Selvitys koulutuksen tai kokemuksen kautta hankitusta riittävästä ammattitaidosta

[redacted]

Liitteenä CV (Liite 5).

5.4. Kirjallinen tehtävänkuva ja sijainti organisaatiossa

[redacted]

5.5 Ote konkurssi- ja yrityssaneerausrekisteristä

Liitteenä 6.

5.6 Ote holhousasioiden rekisteristä

Liitteenä 7.

6 Tiedot vara-vastuuhenkilöstä (16 §):

6.1 Vara-vastuuhenkilön nimi ja henkilötunnus:

[redacted] Sotu: [redacted]

6.2 Vara-vastuuhenkilön yhteystiedot:

[redacted]

6.3 Selvitys koulutuksen tai kokemuksen kautta hankitusta riittävästä ammattitaidosta

[redacted]
Liitteenä CV (Liite 8).

6.4 Kirjallinen tehtävänkuva ja sijainti organisaatiossa

[redacted]

6.5 Ote konkurssi- ja yrityssaneerausrekisteristä

Liite 9.

6.6 Ote holhousasioiden rekisteristä

Liite 10.

7 Tiedot vara-vastuuhenkilöstä (16 §):

7.1 Vara-astuuhenkilön nimi ja henkilötunnus:

[redacted] Sotu: [redacted]

7.2 Vara-vastuuhenkilön yhteystiedot:

[redacted]

7.3 Selvitys koulutuksen tai kokemuksen kautta hankitusta riittävästä ammattitaidosta

[redacted]

CV liitteenä 11.

7.4 Kirjallinen tehtävänkuva ja sijainti organisaatiossa

[redacted]

7.5 Ote konkurssi- ja yrityssaneerausrekisteristä

Liite 12.

7.6 Ote holhousasioiden rekisteristä

Liite 13.

LIITTEET

Liite 1. Aalto yliopiston organisaatiokaavio

Liite 2. Kemian tekniikan korkeakoulun organisaatiokaavio

Liite 3. Kirjallinen vakuutus.

Liite 4. Reaktiotaulukko.

Liite 5. [redacted] CV.

Liite 6. [redacted] Ote konkurssi- ja yrityssaneerausrekisteristä.

Liite 7. [redacted] Ote holhousasioiden rekisteristä.

Liite 8. [redacted] CV.

Liite 9. [redacted] Ote Konkurssi- ja yrityssaneerausrekisteristä.

Liite 10. [redacted] Ote holhousasioiden rekisteristä.

Liite 11. [redacted]

Liite 12. [redacted] Ote Konkurssi- ja yrityssaneerausrekisteristä.

Liite 13. [redacted] Ote holhousasioiden rekisteristä.

Appendix 2: Censored manufacturing permit application for Amphetamine in Finnish

Huumausaineiden valmistuslupahakemus (373/2008 12 §)

1 Organisaation tiedot

Aalto-yliopisto
Kemian tekniikan korkeakoulu
Y-Tunnus: [redacted]
Organisaatio kaavio Liitteessä 1

Postiosoite
Aalto-yliopisto
Kemian tekniikan korkeakoulu
PL 16100
00076 AALTO

Käyntiosoite
[redacted]

Kemian tekniikan korkeakoulu
Kemian Laitos
Organisaatiokaavio Liitteessä 2

Postiosoite
PL 16100
00076 AALTO

Käyntiosoite
[redacted]

2 Valmistettavan huumausaineen nimi ja määrä (373/2008 3§ momentti 5b)

Amfetamiini: [redacted]

3 Valmistettavan huumausaineen käyttötarkoitus

Amfetamiinin valmistus on välttämätöntä kyseisen aineen synteettisen epäpuhtausprofiilin tutkimiseen. Tutkimus liittyy ”Luonnonainetutkimus”-nimiseen projektiin. Tutkimuksen kohteena on eri synteesireittejä pitkin syntyvät karakteristiset epäpuhtaudet, joiden avulla voidaan päätellä huumausaineen alkuperä. Tämän vuoksi kyseistä ainetta on valmistettava eri synteesireittejä pitkin, sellaisia määriä, että myös kiinnostavat epäpuhtaudet, joita voidaan olettaa syntyvän noin 1-5% synteesin päätuotteesta voidaan eristää ja karakterisoida. Yhden synteesin aikana tehdään enintään [redacted] grammaa amfetamiinia ja sitä säilötään vain niin kauan, että se saadaan analysoitua. Tämän vuoksi varastoitua amfetamiinia on kerralla enintään [redacted] Tehtävät synteesit ovat eritelty liitteessä 4.

Vastuuhenkilön allekirjoitus ja päivämäärä Vara-vastuuhenkilön allekirjoitus ja päivämäärä

Appendix 2: The answer to the request for addition information from FIMEA

Hyvä vastaanottaja,

Tässä viestissä on esitetty selvennyksiä ja tarkennuksia aiemmin lähetettyihin Aalto yliopiston kemiantekniikan korkeakoulun käsittely- ja valmistuslupahakemuksiin amfetamiinin ja fenyyliasetonin osalta, niiltä osin, kun niitä on pyydetty 18. Helmikuuta 2016 lähetetyssä lisäselvityspyynnössä. Lisäksi tämän viestin liitteenä on APAAN:in käsittelylupahakemus, johon myöskin pätee tässä viestissä esitetyt selvennykset ja tarkennukset.

Lääkealan turvallisuus- ja kehittämiskeskuksella on käsittelyssä Aalto-yliopiston Kemian tekniikan korkeakoulun kemian laitoksen huumausaineen käsittely- ja valmistuslupahakemukset sekä huumausaineen lähtöaineen käsittelylupahakemus:

- Huumausaineen käsittelylupa, dnro [redacted].

- Huumausainelain mukaisen vastuuhenkilön hyväksyminen , dnro [redacted]

- Huumausaineen valmistuslupa, dnro [redacted]. ja

- Huumausaineen lähtöaineen käsittelylupa, dnro [redacted]..

Pyydämme selvitystä siitä tieteellisestä tutkimuksesta ja sen tavoitteista ja tarpeellisuudesta, johon luvanvaraisten aineiden käsittely liittyy.

Liitteenä on lähetetty tutkimussuunnitelma (”tutkimussuunnitelma suomeksi 22022016.docx”), jossa ilmenee tieteellisen tutkimuksen tavoitteet ja tarpeellisuus.

3.1 Huumausaineluvan alaisten toimintojen laatu ja laajuus

- Pyydämme perusteellisempaa selvitystä valmistettavasta määrästä amfetamiinia. Mihin perustuu ilmoittamanne arvio valmistettavasta amfetamiinimäärästä, tarvitsemme tästä tarkemman selvityksen? Huumausaineen valmistuslupa voidaan myöntää enintään kalenterivuodeksi. Pyydämme arvioimaan miten nämä määrät tulevat valmistetuiksi (synteesit, arvioidut saannot, kulutetut lähtöaineet), kulutetuiksi ja hävitetyiksi ajallisesti luvan myöntämisestä eteenpäin. Tarkennukset koskevat kaikkia luvanvaraisia aineita.

Liitteenä lähetetystä tutkimussuunnitelmasta ilmenee tarkemmin miksi meidän on tehtävä kyseinen määrä amfetamiinia ja miksi meidän on käytettävä C^{13} NMR-analyysiä yhtenä analyysimenetelmänä. Lisäksi liitteenä olevasta päivitetystä reaktiotaulukosta (”Liite 4 reaktiotaulukko 22022016.xlsx”) ilmenee kuinka kauan yhden reaktiopolun aineita (sekä lähtöaineita että tuotteita) käsitellään, ennen kun ne tuhotaan.

3.2.3 Huumausaineiden luovuttaminen varastosta ja kirjaaminen varastoon:

- Kuvatkaa dokumentointi tarkemmin.

Huumausaineita luovutetaan varastoon ja kirjataan varastoon niin, että kaksi henkilöä on aina läsnä ja heidän allekirjoituksensa merkitään huumausainekirjanpitoon. Kirjanpitoon merkitään myös luovutetun huumausaineen nimi ja säilytysastian koodi, luovutetun huumausaineen määrä, henkilön nimi jolle huumausaine on luovutettu, jäljellä olevan huumausaineen määrä, huumausaineen käyttötarkoitus (eli, mihin tutkimuskäyttöön huumausainetta luovutetaan, tai se, että se poistetaan varastosta tuhottavaksi), päivämäärä toimenpiteelle sekä huumausaineen hankinta tai tekopaikka ja päivämäärä. Vain hakemuksen kohdassa 5, 6 ja 7 merkityt huumausaineen vastuuhenkilöt voivat luovuttaa huumausaineita varastosta tai kirjata niitä varastoon. Yhden nimikirjoituksista on siis oltava vastuu- tai varavastuuhenkilön ja toinen nimikirjoitus, henkilön jolle huumausaine on luovutettu.

- Mitä tarkoitatte ”yhtenäisellä käytännöllä” luovutusten ja varastoinnin yhteydessä?

Yhtenäisellä käytännöllä viitataan tässä lisäselvityspyynnössä ja aiemmin lähetetyssä hakemuksessa kuvattuun käytäntöön, jota noudatetaan joka kerta kun huumausainetta luovutetaan tai kirjataan varastoon, eli yllä (kohta 3.2.3) kuvattuun kirjanpitokäytäntöön.

- Miten tilapäinen luovuttaminen kuitataan vastaanotetuksi ja palautetuksi, paljonko tutkijoille ainetta luovutetaan ja tutkitaanko palautuvia aineita? Pyydämme arvioimaan kulloinkin tarvittavat määrät tarkemmin sekä nimeämään mahdollisimman tarkoin ne henkilöt, jotka on oikeutettuja ainetta käsittelemään.

Tilapäinen luovutus tutkijalle kuitataan luovutetuksi ja vastaanotetuksi huumausainekirjanpitoon yllä kuvatun käytännön mukaisesti, eli niin, että kirjanpitoon merkitään sekä luovuttavan vastuu- tai varavastuuhenkilön nimikirjoitus että vastaanottajan nimikirjoitus, ja yllä kuvatut (3.2.3) muut tiedot. Huumausainetta luovutetaan enintään se määrä, jota tutkija tarvitsee päivän työskentelyyn, eli tämän tutkimuksen puitteissa, enintään [redacted] amfetamiinia. Huumausaineiden lähtöaineita luovutetaan myös enintään päivän työskentelyyn tarvittava määrä, liitteenä olevan reaktiotaulukon mukaisesti.

Luvanvaraisia aineita luovutetaan ainoastaan luotettaville ja päteville tutkijoille, jotka pystyvät työskentelemään yleisen laboratorioturvallisuuden ja aineen vaatiman varovaisuuden mukaisesti. Ymmärtääksemme heidän yksittäinen nimeäminen ei kuitenkaan ole lupamenettelyn edellytys, luvan myöntämisen peruste, eikä heitä voi asettaa vastuuseen muusta kun heille henkilökohtaisesti ja tilapäisesti luovutetusta luvanvaraisesta aineesta. Kenelle, koska ja kuinka paljon luvanvaraista ainetta on luovutettu tilapäiseen käyttöön, selviää yksiselitteisesti huumausainekirjanpidosta. Näissä hakemuksissa nimetyt vastuu- ja varavastuuhenkilöt arvioivat kenelle huumausaineita luovutetaan tilapäisesti ja he ovat vastuussa siitä, että luvanvaraisia aineita käytetään ainoastaan tämän hakemuksen mukaiseen tieteelliseen tutkimuskäyttöön. Tämän tutkimuksen yhteydessä luvanvaraisia aineita käsittelee enintään kolme tutkijaa. Mikäli olette sitä mieltä, että luvan saamisen edellytys on myös luvanvaraisia aineita tilapäisesti käsittelevien henkilöiden nimeäminen, toivomme että palaatte asiaan.

- Tullaanko luovutettua ainetta säilyttämään ilman tutkijan läsnäoloa laboratoriotiloissa, ovatko nämä tilat lukittuja ja palautuuko aine aina huumausainevarastoon yöksi?

Huumausaineita ei ikinä säilytetä yön yli laboratoriossa (liuoksessa tai muutenkaan), vaan ne palautetaan aina huumausainevarastoon yöksi. Työpäivän aikana luovutettuja aineita ei säilytetä puhtaina laboratoriossa ilman tutkijan välitöntä läsnäoloa. Laboratoriotilat ovat lukittuja ja niihin on pääsy [redacted] Luvanvaraisia huumausaineita valmistettaessa, tutkija voi poistua vetokaapin välittömästä läheisyydestä lyhyeksi aikaa, mutta silloin huumausaineet ovat liuoksissa muiden aineiden kanssa, joista niitä ei saada käyttöön ilman kemian osaamista ja laboratoriolaitteistoa, eikä niitä voida ns. ”laittaa taskuun” nopeasti.

3.3 Huumausaineiden hävityskäytännöt

- Miten hävitys dokumentoidaan (vrt. huumausainelainsäädäntö: hävityspöytäkirja ja kahden henkilön läsnäolo)

Huumausaineita hävitettäessä pidetään hävityspöytäkirjaa (erillinen huumausainekirjanpidosta). Läsnä on aina kaksi henkilöä, joista toinen on näissä hakemuksissa mainittu vastuu- tai varavastuuhenkilö. Huumausaineiden hävityspöytäkirjaan merkitään: ketkä aineen on hävittänyt (kaksi henkilöä), kuinka paljon ainetta on hävitetty, mitä ainetta on hävitetty, mistä säilytyspullosta hävitettävä aine on otettu, mihin liuottimeen hävitettävä aine on liuotettu, mihin jäteastiaan hävitettävä aine on laitettu ja koska hävitys on tehty. Tämä lisäksi tehdään myös huumausainekirjanpitoon merkintä, josta ilmenee aiemmassa kohdassa (3.2.3) ilmoitetut asiat. Hävityspäiväkirja tehdään samanlaiseen laboratoriopäiväkirjaan kun huumausainekirjanpito (kts. Täsmennys alla, 3.4 huumausainekirjanpidon kuvaus).

- Mikä on ilmoittamanne sopiva liuotin?

Sopiva liuotin valitaan niin, että myös kaikki epäpuhtaudet liukenevat liottimeen. Arvioimme että diklorometaani toimii useimmissa tapauksissa. Myös muita aineen täydellisesti liuottavia yleisiä orgaanisia liuottimia voidaan tarpeen mukaan käyttää (mikäli diklorometaani ei liuota kaikkia aineseoksessa olevia aineita).

3.4 Huumausainekirjanpidon kuvaus

- Mitä tarkalleen tarkoitatte termillä ”tarkoituksenmukainen” laboratoriopäiväkirja?

Tarkoituksenmukaisella laboratoriopäiväkirjalla tarkoitamme kovakantista A4 kokoista konttorikirjaa. Merkinnät tehdään kuulakärkikynällä. Samanlaista laboratoriopäiväkirjaa ja merkintätapaa käytetään laboratorion muussa toiminnassa, jotta esimerkiksi patenttihakemuksissa tai muissa selvitystä vaativissa tilanteissa voidaan osoittaa koska, kuka ja mitä on tehty.

Lääkealan turvallisuus- ja kehittämiskeskus voi kirjata lupapäätökseen erityisiä ehtoja, mikäli asia katsotaan tarpeelliseksi.

Olette hakenut huumausaineen käsittelylupaa amfetamiinin käsittelemiseksi ja valmistamiseksi. Lisäksi olette hakenut fenyyliasetonin käsittely- ja valmistuslupaa. Fenyyliasetoni on luokiteltu huumausaineen lähtöaineeksi, tarkemmin luokkaan 1 kuuluvaksi ja sen käsittely (valmistus, hankkiminen, varastointi) edellyttää huumausaineen lähtöaineen käsittelylupaa. Toimittamassanne synteesitaulukossa on amfetamiinin ja fenyyliasetonin lisäksi mainittu myös APAAN (alfa-fenyyliasetoasetonitriili), joka on myös luokan 1 huumausaineiden lähtöaine ja sen käsittely edellyttää lupaa samoin kuin fenyyliasetoni.

Liitteenä on APAAN:in käsittelylupahakemus. Siihen pätee samat tässä esitetyt selvennykset ja tarkennukset kun aiemmin lähetettyihin käsittely- ja valmistuslupahakemuksiin.

Lähtöaineiden käsittelylupaa varten tarvitsemme vielä huumausaineiden lähtöaineista vastaavan henkilön nimen ja hänen yhteystietonsa.

Kaikissa lähtöaineiden ja huumausaineiden käsittelylupahakemuksissa (fenyyliasetoni, amfetamiini ja APAAN) on merkitty vastuu- ja varavastuuhenkilöt (kohdat 5, 6 ja 7), heidän yhteystietonsa ja vaadittavat rekisteriotteet on lähetetty liitteinä.

Pyydän tutustumaan huumausainelainsäädäntöön, linkit lakiin ja asetuksiin ovat verkkosivuillamme http://www.fimea.fi/valvonta/huumausainevalvonta .

Pyydetyt lisäselvitykset tulee toimittaa Lääkealan turvallisuus- ja kehittämiskeskuksen kirjaamoon yhden (1) kuukauden kuluessa. Määräajan noudattamatta jättäminen ei estä asian ratkaisemista, mutta määräaikaa voidaan erillisestä pyynnöstä pidentää, jos se on tarpeen asian selvittämiseksi.

Toivomme näiden lisätietojen selventävän lupahakemusta riittävästi ja toivomme, että olette mahdollisten lisäkysymysten osalta yhteydessä mahdollisimman pian. Mikäli teillä ilmenee toiveita tai muita kommentteja erityisesti käsittely, luovutus ja kirjanpitokäytännöistä noudatamme niitä mielellämme.

Ystävällisin terveisin,

[redacted]

Appendix 4: Research plan from 22nd of February 2016

Synteettinen epäpuhtausprofiili Amfetamiinille, joka on tehty neljästä eri lähtöaineesta valmistetusta P-2-P:sta

Tutkimussuunnitelma, Päivitetty 22.2.2016

Tausta

Rikostekninen tutkimus (Forensic science) on perinteisesti tuottanut todistusaineistoa juridista prosessia varten. Nykyään tiedostetaan rikosteknisen tutkimuksen tuottaman tiedon tärkeys myös muissa yhteyksissä, mm. esitutkinnan ohjaamisessa, rikosteknisessä tiedustelussa (forensic intelligence) ja yleisen tilannekuvan luomisessa. Laittomien huumausaineiden osalta tämä merkitsee sitä, että takavarikoitujen huumeiden alkuperästä halutaan tietää mm. missä, koska ja miten ne on tehty. Tämä toteutetaan nk. epäpuhtausprofiilin avulla, joka parhaimmassa tapauksessa on spesifi kaikkien edellä mainittujen kysymysten osalta. Vaikka tämä tieto olisi periaatteessa olemassa, haasteena on usein myös tiedon saatavuus. Tämän vuoksi on käynnistetty useita eurooppalaisia projekteja, kuten Collaborative Harmonizing of Methods for Profiling Amphetamine Type Substances (CHAMP) ja The European Drug Profiling System (EDPS) huumeiden profilointimenetelmien harmonisoimiseksi ja tiedon jakamisen helpottamiseksi eri poliisiviranomaisten välillä.[1]

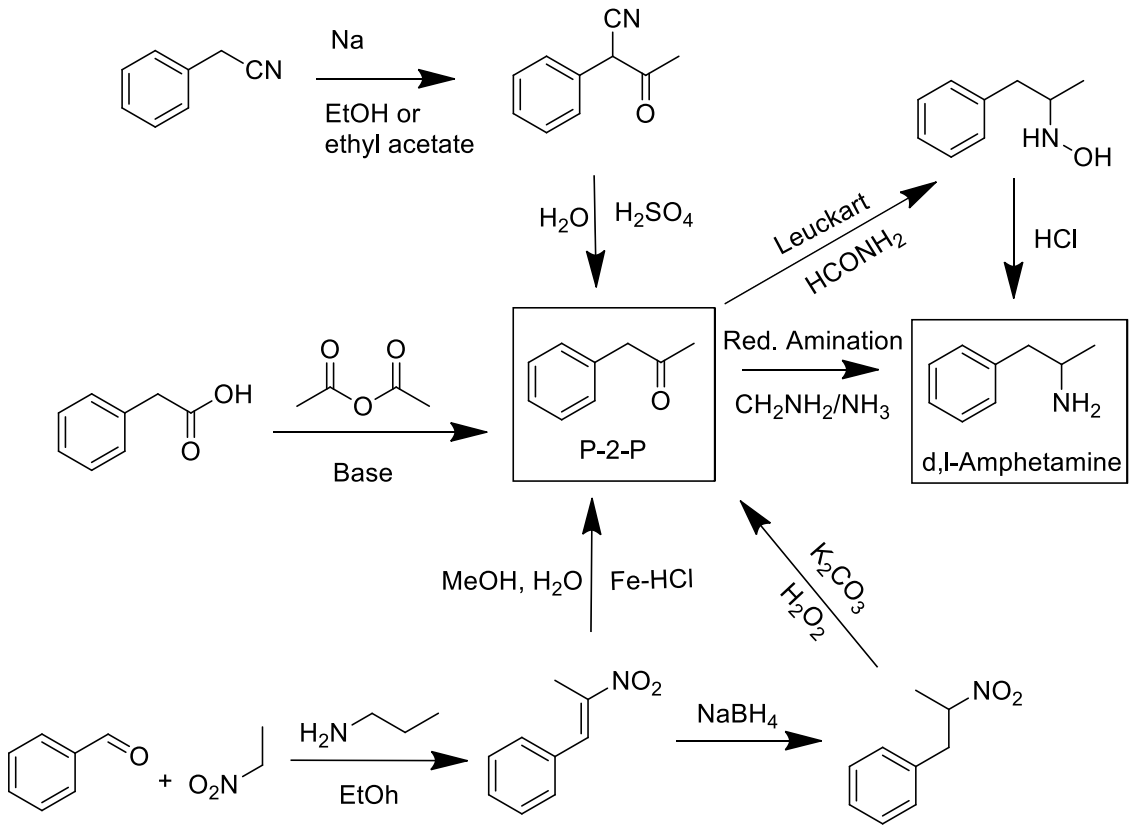
Stojanovskan ynnä muiden julkaisema kirjallisuuskatsaus amfetamiinityyppisten huumeiden (ATS) synteesi- ja profilointimenetelmistä toteaa, että suurin osa profilointiin liittyvistä artikkeleista keskittyy profiilien keskinäiseen vertailuun, koska usein ollaan kiinnostuneita siitä, ovatko kaksi eri huumetakavarikkoa peräisin samasta lähteestä. Profiileista voitaisiin kuitenkin myös identifioida synteesireiteille ominaisia epäpuhtauksia ja niiden avulla todeta mitä lähtöaineita, synteesimenetelmää, tieto-taitoa ja laboratoriolaitteistoa on käytetty.[2]

Tämä työ

Amfetamiini on Euroopan kolmanneksi eniten käytetty huume ja valtaosa siitä valmistetaan Euroopassa fenyylifenoni (P-2-P) nimisestä lähtöaineesta, joko Leuckart-nimistä synteesireittiä pitkin tai pelkistävällä aminoinnilla. ”Amphetamine – A European Union Perspective in a Global Context”[3] luettelee myös P-2-P:n yleisimmät valmistusreitit.

Sekä amfetamiinin että P-2-P epäpuhtausprofiileita on tutkittu paljon, mutta näitä kahta epäpuhtausprofiilia ei ole yhdistetty (J. D. Power, 2014[4] on yksi harvoista esimerkeistä, APAAN-P2P-Leuckart). Tässä työssä tehdään systemaattinen tutkimus siitä, miten P-2-P:n eri valmistusreittejä pitkin syntyvät epäpuhtaudet näkyvät valmiissa amfetamiinissa. Tämä toteutetaan käytännössä niin, että ensin syntetisoidaan P-2-P:ta eri lähtöaineita käyttäen ja analysoidaan mitä muita tuotteita, eli epäpuhtauksia kutakin reittiä pitkin muodostuu. Sen jälkeen annetaan edellisessä vaiheessa syntetisoidut P-2-P erät reagoida edelleen amfetamiiniksi kahdella eri menetelmällä (pelkistävä aminointi ja Leuckart). Tällöin P-2-P muuttuu amfetamiiniksi, mutta myös kaikki P-2-P:n synteesissä muodostuneet epäpuhtaudet reagoivat edelleen joksikin muiksi epäpuhtauksiksi. Nämä ovat niitä epäpuhtauksia, jotka löytyvät takavarikoidusta amfetamiinista ja sen takia ne analysoidaan lopuksi ja yhdistetään takaisin P-2-P valmistuksessa käytettyihin lähtöaineisiin. Näin voidaan sanoa mikä on takavarikoidun amfetamiinin synteettinen alkuperä. Tutkittavat synteesireitit on esitetty kuvassa 1. Jokainen synteesi suoritetaan yleisen käytännön mukaisesti kaksi kertaa tulosten toistettavuuden varmistamiseksi. Synteesireitit, tarvittavat lähtöaineet, odotetut saannot ja aineen käsittelyaikataulu on esitetty tarkemmin tiedostossa ” Liite 4

reaktiotaulukko päivitetty.xlsx”.



Kuva 1. 2-P-2:n yleisimmät valmistusreitit ja P-2-P: konversio amfetamiiniksi pelkistävällä aminoinnilla ja Leuckart reittiä pitkin.

Tämän työn vaatimat synteesit tehdään [redacted]. Aalto-yliopiston kemiantekniikan korkeakoulu on hakenut FIMEA:lta lupaa tämän tutkimuksen tekemiseen ja lupahakemuksen käsittely on paraikaa käynnissä.

Analyysi

Useimmat olemassa olevat epäpuhtausprofiilimenetelmät ATS-tyyppisille huumeille tehdään erilaisilla kaasukromatografisilla (GC) tai nestekromatografisilla (LC) menetelmillä. Paras analyysimenetelmä arvioidaan useamman eri kriteerin perusteella. Esimerkiksi, Andersson ynnä muut vertailivat eri GC-detektoreiden sisäistä ja ulkoista tarkkuutta, havaitsemisrajaa, selektiivisyyttä ja lineaarisuutta kehittäessään harmonisoitua menetelmää amfetamiinin epäpuhtausprofiilin tekemiseen.[5] He vertailivat myös tilastollisia

menetelmiä, joilla voidaan tutkia epäpuhtausprofiilien samankaltaisuutta keskenään ja verrattuna referenssi-epäpuhtauksiin, sekä datan (tilastollisia) esikäsittelymenetelmiä. He pystyivät erottamaan eri menetelmillä syntetisoituja (Leauckart, pelkistävä aminointi ja Nitrostyreeni) amfetamiininäytteitä toisistaan ja osoittivat myös, mitkä samalla menetelmällä syntetisoiduista näytteistä olivat peräisin samasta erästä.[6]

Tässä työssä tutkitaan miten P-2-P synteesissä syntyneet (tunnetut tai tuntemattomat) epäpuhtaudet reagoivat eteenpäin kun P-2-P muunnetaan amfetamiiniksi. Sen takia on odotettavissa, että valmiissa amfetamiinissa on P-2-P:n eri synteesireiteille spesifejä epäpuhtauksia, joiden rakennetta ei voida määritellä yksiselitteisesti UPLC-TOFL/MS laitteella (UPLC-TOFL/MS antaa vain isotooppisten massojen perusteella arvauksen rakenteen molekyylikaavasta). Rakenteen yksiselitteiseen määrittämiseen on käytettävä C^{13} ja H^{1} NMR-spektroskopiaa. Tämä on yleinen käytäntö tuntemattomien aineiden rakenteen määrittämisessä niin synteettisessä kemiassa yleensä kun rikosteknisessä huumeanalyysissä. Esimerkiksi Gallagher ynnä muut karakterisoivat näin MDMA:sta löytyneet epäpuhtaudet jotka viittasivat siihen, että MDMA oli tehty joko vaniliinista tai piperiinistä riippuen epäpuhtaudesta.[7] Myös aiemmin mainittu J.D. Power[4] on eristänyt ja karakterisoinut 4,6-dimetyyli-3,5-difenyylipyridin-2-oonin NMR:llä osana täydellistä amfetamiinisynteesiä, epäillen sitä APAAN-reitille ominaiseksi epäpuhtaudeksi.

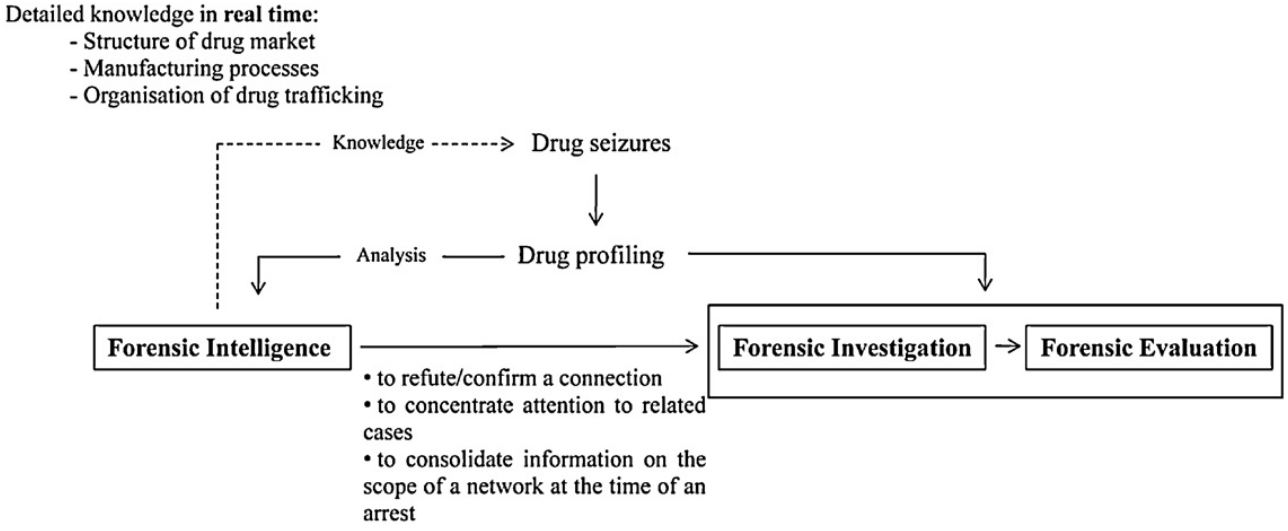
Kunalan ynnä muut karakterisoivat reittispesifejä epäpuhtauksia Methyliamfetamiinista:sta, joka oli tehty Leuckart-menetelmällä ja pelkistävällä aminoinnilla (samat menetelmät, joita tässä tutkimuksessa käytetään muuntamaan P-2-P amfetamiiniksi). Kiinnostavia epäpuhtauksia syntyi heidän tutkimuksessa prosentin suuruusluokassa suhteessa syntetisoituun Methyyliamfetamiinista:han.[8] Tämä on yhteneväistä van der Ark ynnä muiden tuloksiin amfetamiinista löytyvien heikosti emäksisten epäpuhtauksien konsentraatiosta (n. 1-5%) suhteessa syntetisoituun amfetamiiniin.[9] Tuntemattoman aineen rakenteen määrittämiseksi tarvittava C^{13} NMR analyysi vaatii puolestaan 20-30 mg tutkittavaa ainetta (Bruker 400 NMR-laitteella). Tämän vuoksi tässä tutkimuksessa tehdään [redacted]

gramma amfetamiinia kutakin tutkittavaa synteesireittiä pitkin, jotta kiinnostavia epäpuhtauksia saadaan riittävästi (20-30 mg) C^{13} NMR-analyysia varten.

Tämän työn analyysit tehdään Aalto-yliopiston kemian laitoksen UPLC-TOFL/MS laitteella, jonka erotuskyky on tavallisia GC ja LC laitteita parempi (käytössä on käänteisfaasikolonni). Mikäli epäpuhtauden rakenne on osoitettava tai varmistettava, se eristetään pylväskromatografisin menetelmin (tai syntetisoidaan erikseen, mikäli rakenne voidaan yksiselitteisesti määrittää pelkästään UPLC-TOFL/MS spektrin avulla) ja analysoidaan Aalto-yliopiston Kemian laitoksen käytössä olevalla Bruker 400 NMR-laitteella.

Rikostiedustelu

Tämän ja muiden rikostiedustelua tukevien tutkimusten perimmäinen tavoite on tuoda lisäarvoa tiedusteluprosessiin. Kuten Morelato ynnä muut toteavat, tiedusteluprosessi on iteratiivinen, eli se vaatii jatkuvaa interaktiota tiedon tuottajien, analysoijien ja käyttäjien välillä, ja siinä pyritään muuttamaan raakadataa päätöksentekoprosessia tukevaksi tiedustelutiedoksi mahdollisimman pienellä viiveellä.[1] He kiteyttävät huumeprofiloinnista saadun tiedustelutiedon käytön kuvan 2 mukaisesti. Tämän vuoksi tässä tutkimuksessa huomioidaan tutkimustulosten lopullisten käyttäjien tarpeet tutkimuksen suunnittelussa, sen tutkimuskohteen ja menetelmien valinnassa, sekä lopputulosten tuoman lisäarvon arvioinnissa.



Kuva 2. Huumeprofiloinnista saadun tiedon käyttö osana rikostiedustelu- ja –tutkintaprosessia.[1]

Käytännössä tämän tutkimuksen tuoma lisähyöty rikostiedusteluprosessille voidaan tulevaisuudessa arvioida, esimerkiksi väitöskirjatutkimuksessa, tapaustutkimuksen avulla, kuten Esseiva ynnä muut tekivät yhteistyössä Sveitsin keskusrikospoliisin kanssa.[9] He profiloivat yhteensä 67 takavarikkoa 37 eritasoiseen luokkaan ja pystyivät osoittamaan yhteyksiä, joita rikostutkijat eivät aiemmin olleet epäilleet. Suurin hyöty profiloinnista saadusta tiedustelutiedosta oli kuitenkin se, että se voitiin yhdistää muita kanavia pitkin saatuun tiedustelutietoon. Käytännössä siis huumeiden profilointi tuki rikostiedusteluprosessia kahdella tavalla: 1) Se tarjosi vahvistuksen muun tiedustelun antamaan osviittaan, ja 2) se antoi osviittaa siitä, mihin tiedustelu- ja/tai tutkimusresursseja kannattaisi seuraavaksi panostaa. Tämän tutkimuksen tulosten osalta sama voitaisiin tulevaisuudessa toteuttaa yhteistyössä KRP:n tutkimus- ja/tai tilannekuvaosaston kanssa, joko aktiivisen tai jo päättyneen tutkimuksen osalta.

Lopputulokset

Tässä tutkimuksessa lähdetään liikkeelle rikostiedustelun tarpeista ja hyödynnetään synteettisten huumeiden ”sormenjälkiä” mahdollisimman arvokkaan lisätiedon tuottamiseksi. Tämä tutkimus tuottaa myös uutta ja julkaistavaa tieteellistä tietoa, sillä tämän kaltaista ”kaksi askelta taaksepäin” katsovaa systemaattista

epäpuhtausprofiilia ei ole amfetamiinille ennen tehty. Vaikka tämän tutkimuksen laajuus ei anna mahdollisuutta arvioida lisäarvoa systemaattisesti, eli synteettisen epäpuhtausprofiilikirjaston ja useamman eri rikostutkimuksen kautta, se olisi kuitenkin yksi esimerkki menetelmän toimivuudesta.

Väitöskirjatutkimuksessaan Christian Zingg luokitteli satojen ekstaasi tablettien kemialliset ja fysikaalista profiilit ja validoi löydetyt yhteydet aktiivisissa rikostutkimuksissa.[10] Vastaava olisi tulevaisuudessa mahdollista myös synteettisten sormenjälkien osalta, tulevassa väitöskirjatutkimuksessa.

Aikataulu

Syksy (syyskuun alusta joulukuun puoleen väliin) 2015 käytettiin lupahakemuksen tekemiseen ja se lähetettiin FIMEA:lle joulukuun puolessa välissä. FIMEA:lta odotetaan päätöstä maaliskuun aikana, jolloin laboratoriotyöt päästään aloittamaan viimeistään huhtikuun alussa. Synteesiin ja analyysiin arvioidaan menevän neljä kuukautta (huhti-, touko, kesä- ja heinäkuu) ja kirjoittamiseen kaksi kuukautta (elo- ja syyskuu). Katso tarkempi aikataulu tiedostosta ”Liite 4 reaktiotaulukko päivitetty.xlsx”.

Uusia reaktioita tehdään yksi per viikko, ne analysoidaan lähtökohtaisesti seuraavalla viikolla ja tuhotaan kolmannella viikolla. Mikäli mitään viivästyksiä ei synny, tehtyä [redacted] amfetamiinierää säilötään siis enintään [redacted], jonka jälkeen se tuhotaan. Tämä merkitsisi sitä, että varastossa olisi enintään [redacted] g amfetamiinia kerralla. Ei kuitenkaan voida olettaa etteikö tutkimuksen aikana synny syystä tai toisesta viivästyksiä (laiteviat, sairastumiset, etc) ja sen takia kaikkiin valmistuseriin pitää laskea viikon marginaali. Tämä merkitsee sitä, että valmistettua [redacted] amfetamiini erää säilötään enintään neljä viikkoa ennen kun se tuhotaan ja varastossa on täten enintään [redacted] amfetamiinia kerralla. Lähtöaineiden valmistus, analysointi ja tuhoaminen noudattaa samaa aikataulua ja yksityiskohdat niiden määristä selviää tiedostosta ”Liite 4 reaktiotaulukko päivitetty.xlsx”.

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Appendix 5: The results from the UPLC-TOF-MS analysis

Amphetamine **1** oil from route **I**	Predicted mass [M+H]	Found mass [M+H]	ppm error [M+H]	Retention time (min) [M+H]	Predicted mass [M+Na]	Found mass [M + Na]	ppm error [M+Na]	Retention time (min) [M+Na]
Amphetamine **1**	136,1126	136,113	2,94	0,67				
P2P **5**								
1,3-diphenyl-2-aminopropanone **6**								
1,3-diphenyl-2-aminopropane **3**	212,1436	212,1457	9,90	3,16				
Phenylacetic acid **7**								
Benzylphenylethylamineformamide **4**	240,1388	240,1402	5,83	4,26	262,1208	262,1222	5,34	4,26
APAAN **10**								
Benzyl Cyanide **17**								
4,6-dimethyl-3,5-diphenylpyridine-2-one **9**	276,1388	276,1351	-13,40	4,84	298,1208	298,1222	4,70	4,84
12a or **12b**	260,1439	260,147	11,92	4,51				
1,5-diphenyl-2-methyl-4-oxopent-1-ene **15**					273,1255	273,1273	6,59	5,1
1,5-diphenyl-4-methylpent-1-ene **14**								
Acetylamphetamine **13**	178,1232	178,1237	2,81	3,64	200,1051	200,1066	7,50	3,64
18	254,1545	254,1549	1,57	4,39	276,1364	276,1351	-4,71	4,84
1-phenyl-2-propanol **16**								
Acetic anhydride **8**								

Amphetamine **1** oil from route **II**	Predicted mass [M+H]	Found mass [M+H]	ppm error [M+H]	Retention time (min) [M+H]	Predicted mass [M+Na]	Found mass [M + Na]	ppm error [M+Na]	Retention time (min) [M+Na]
Amphetamine **1**	136,1126	136,1099	-19,84	0,69				
P2P **5**								
1,3-diphenyl-2-aminopropanone **6**								
1,3-diphenyl-2-aminopropane **3**	212,1439	212,1448	4,24	3,13				
Phenylacetic acid **7**								
Benzylphenylethylamineformamide **4**	240,1388	240,1403	6,25	4,21	262,1208	262,1233	9,54	4,21
APAAN **10**								
Benzyl Cyanide **17**								
4,6-dimethyl-3,5-diphenylpyridine-2-one **9**	276,1388	276,1327	-22,09	4,77	298,1208	298,1233	8,39	4,77
12a or **12b**								
1,5-diphenyl-2-methyl-4-oxopent-1-ene **15**								
1,5-diphenyl-4-methylpent-1-ene **14**								
Acetylamphetamine **13**	178,1232	178,1239	3,93	3,63	200,1051	200,1067	8,00	3,63
18					276,1364	276,134	-8,69	4,77
1-phenyl-2-propanol **16**					159,0786	159,0797	6,91	3,8
Acetic anhydride **8**								

Amphetamine **1** oil from route **III**	Predicted mass [M+H]	Found mass [M+H]	ppm error [M+H]	Retention time (min) [M+H]	Predicted mass [M+Na]	Found mass [M + Na]	ppm error [M+Na]	Retention time (min) [M+Na]
Amphetamine **1**	136,1126	136,1119	-5,14	0,69				
P2P **5**								
1,3-diphenyl-2-aminopropanone **6**					233,0942	233,0962	8,58	4,51
1,3-diphenyl-2-aminopropane **3**	212,1439	212,1436	-1,41	3,13				
Phenylacetic acid **7**								
Benzylphenylethylamineformamide **4**	240,1388	240,1396	3,33	2,23	262,1208	262,1227	7,25	2,23
APAAN **10**								
Benzyl Cyanide **17**								
4,6-dimethyl-3,5-diphenylpyridine-2-one **9**	276,1388	276,1377	-3,98	4,34				
12a or **12b**	260,1439	260,1463	9,23	4,5				
1,5-diphenyl-2-methyl-4-oxopent-1-ene **15**								
1,5-diphenyl-4-methylpent-1-ene **14**								
Acetylamphetamine **13**	178,1232	178,1218	-7,86	3,63	200,1051	200,1064	6,50	3,63
18	254,1545	254,1557	4,72	4,34	276,1364	276,1378	5,07	4,34
1-phenyl-2-propanol **16**								
Acetic anhydride **8**								

Amphetamine **1** oil from route **IV**	Predicted mass [M+H]	Found mass [M+H]	ppm error [M+H]	Retention time (min) [M+H]	Predicted mass [M+Na]	Found mass [M + Na]	ppm error [M+Na]	Retention time (min) [M+Na]
Amphetamine **1**	136,1116	136,1118	1,47	0,69				
P2P **5**								
1,3-diphenyl-2-aminopropanone **6**					233,0942	233,0941	-0,43	4,41
1,3-diphenyl-2-aminopropane **3**	212,1439	212,1431	-3,77	3,17				
Phenylacetic acid **7**					159,0422	159,0432	6,29	3,4
Benzylphenylethylamineformamide **4**	240,1388	240,1397	3,75	4,15	262,1208	262,1219	4,20	4,15
APAAN **10**								
Benzyl Cyanide **17**								
4,6-dimethyl-3,5-diphenylpyridine-2-one **9**	276,1388	276,1377	-3,98	4,28				
12a or **12b**								
1,5-diphenyl-2-methyl-4-oxopent-1-ene **15**								
1,5-diphenyl-4-methylpent-1-ene **14**								
Acetylamphetamine **13**	178,1232	178,1235	1,68	3,64	200,1051	200,1064	6,50	3,64
18	254,1545	254,1557	4,72	4,28	276,1364	276,1379	5,43	4,28
1-phenyl-2-propanol **16**								
Acetic anhydride **8**					159,0422	159,0432	6,29	3,4

Appendix 6: The spectra from the UPLC-TOF-MS analysis

Sample JHO-023_050916 corresponds to the amphetamine **1** oil sample from route **I,** sample JHO-029_050916 corresponds to route **II**, sample JHO-013_050916 corresponds to route **III** and sample JHO-031_050916 to route **IV**.

First, the TIC spectra, when searching for the mass of the $[M+H]^+$ and the $[M+Na]^+$ ion is shown. Then the mass spectra from the found peaks at different retentions times is shown, compared to the predicted masses of the $[M+H]^+$ ion and the $[M+Na]^+$ ion (the two spectra at the bottom of the page).

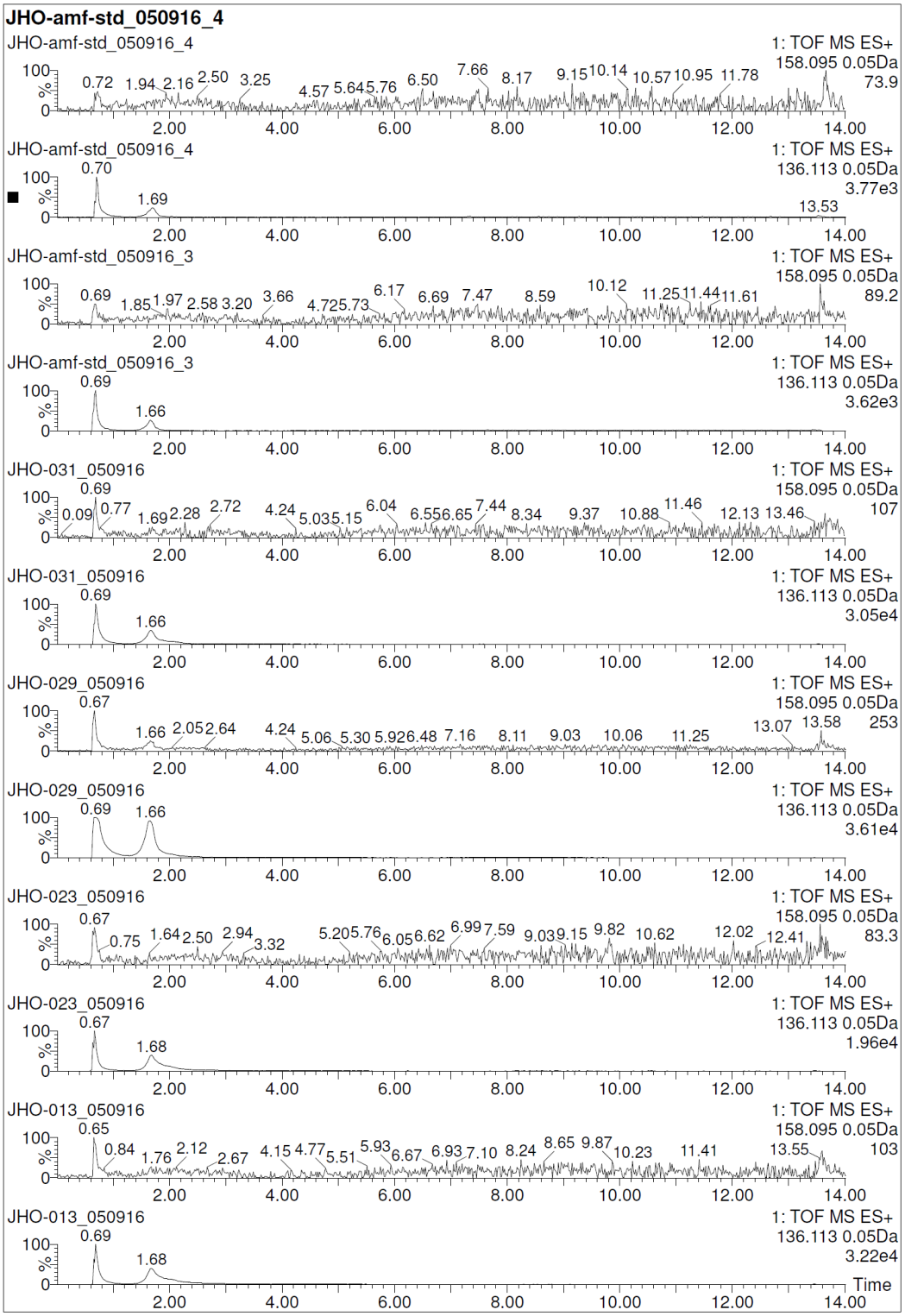
The spectra are presented in the same order as table 4 and 5:

Amphetamine **1**
P2P **5**
1,3-diphenyl-2-aminopropanone **6**
1,3-diphenyl-2-aminopropane **3**
Phenylacetic acid **7**
Benzylphenylethylamineformamide **4**
APAAN **10**
Benzyl Cyanide **17**
4,6-dimethyl-3,5-diphenylpyridine-2-one **9**
12a or **12b**
1,5-diphenyl-2-methyl-4-oxopent-1-ene **15**
1,5-diphenyl-4-methylpent-1-ene **14**
Acetylamphetamine **13**
2-oxo-1-phenyl-(beta-phenylisopropylamino)etane **18**
1-phenyl-2-propanol **16**
Acetic anhydride **8**

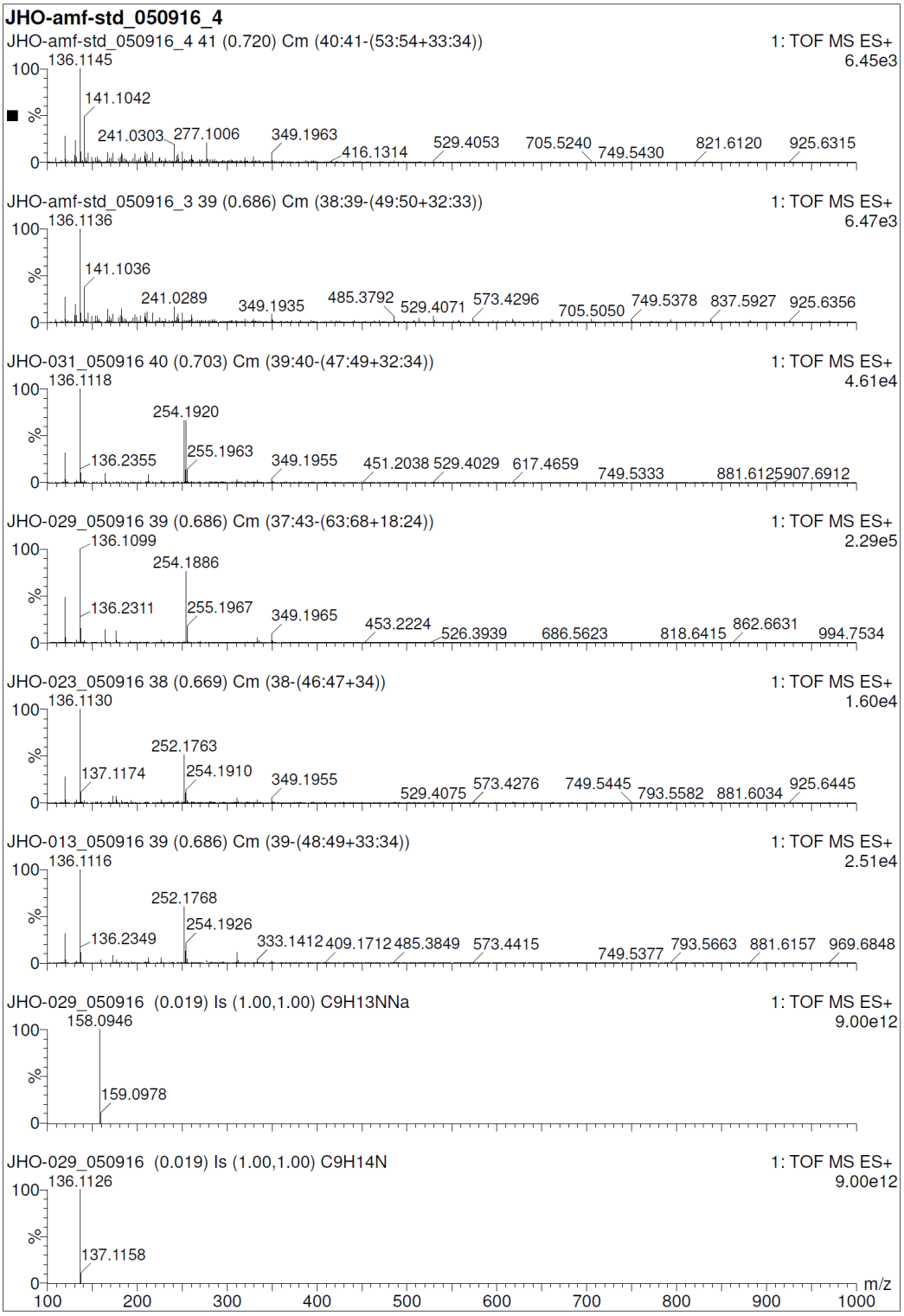
The spectra for the amphetamine reference standard run presented in Table 2 are named JHO-amf-std-150816_1, JHO-amf-std-150816_2, JHO-amf-std-150816_3, etc.

The spectra for the amphetamine reference standard run presented in Table 3 are named JHO-amf-std_050916_3 (before the first experiment-run), JHO-amf-std_050916_3_repeat (before the second experiment-run), JHO-amf-std_050916_4 (after the first experiment-run) and JHO-amf-std_050916_4_repeat (after the second experiment run).

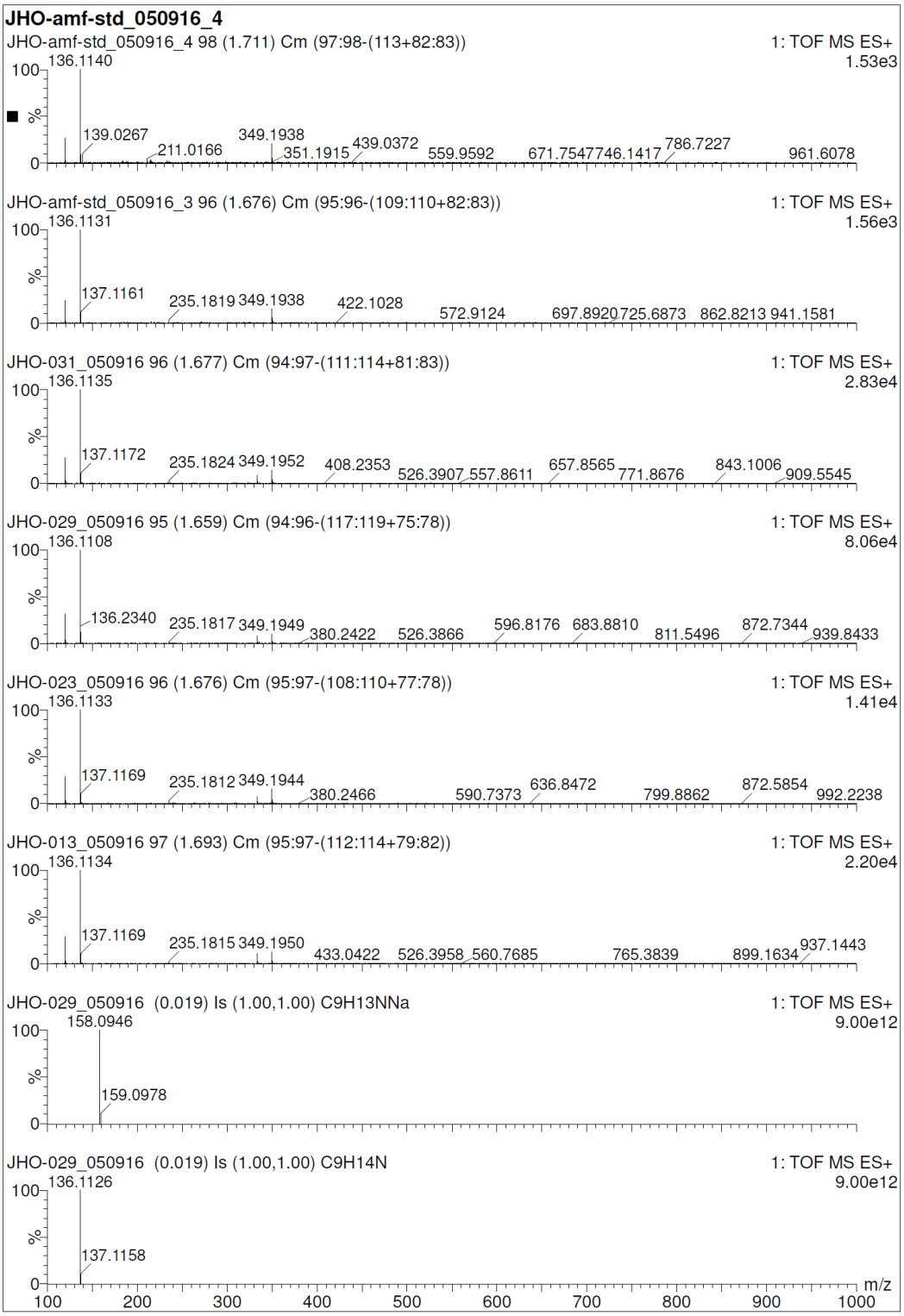
Amphetamine 1, find mass MH MNa TIC, including standard run 3 (before) and 4 (after)



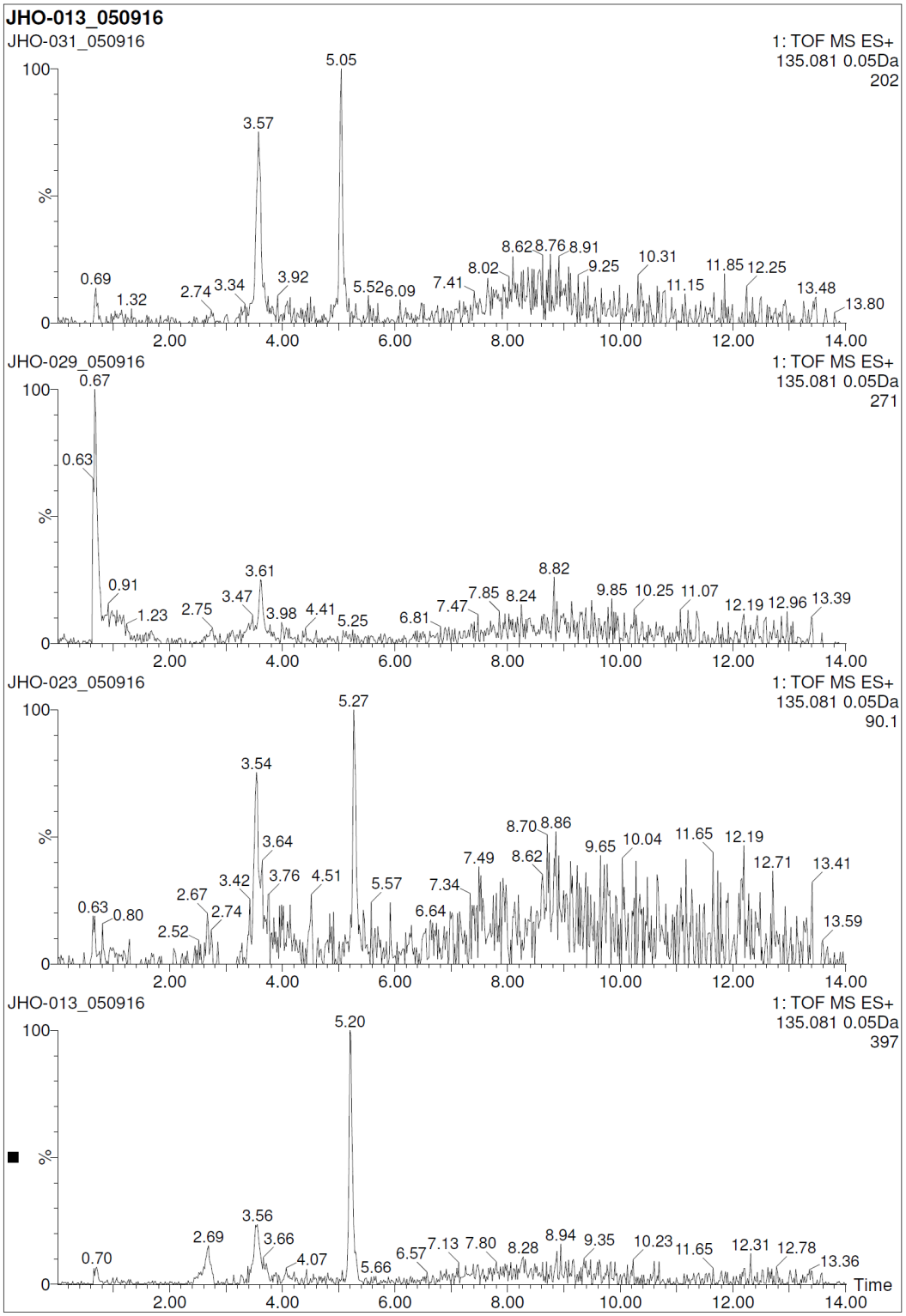
Amphetamine 1 mass for retention time 0,69, including standard run 3 (before) and 4 (after)



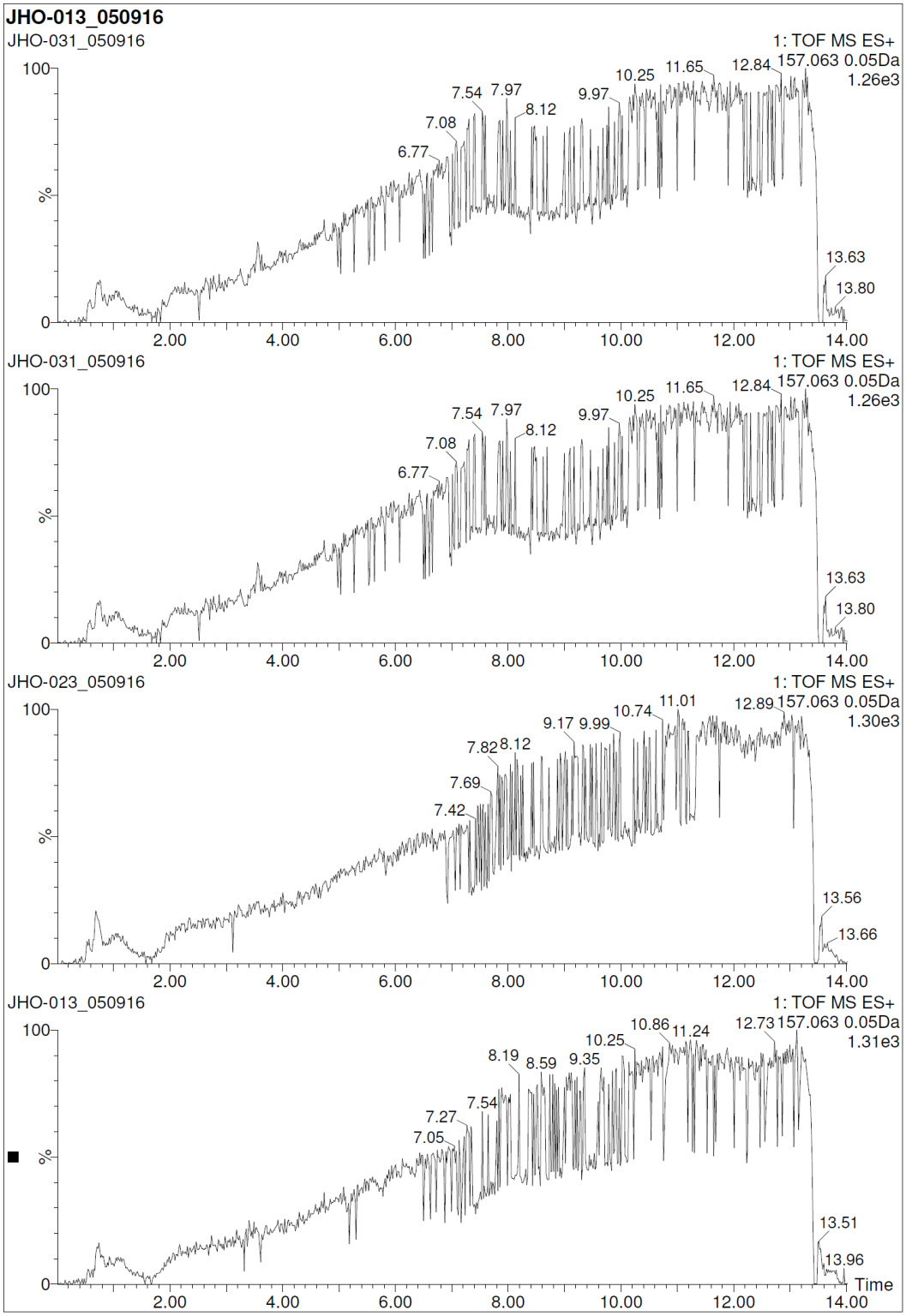
Amphetamine 1 mass for retention time 1,68, including standard run 3 (before) and 4 (after)



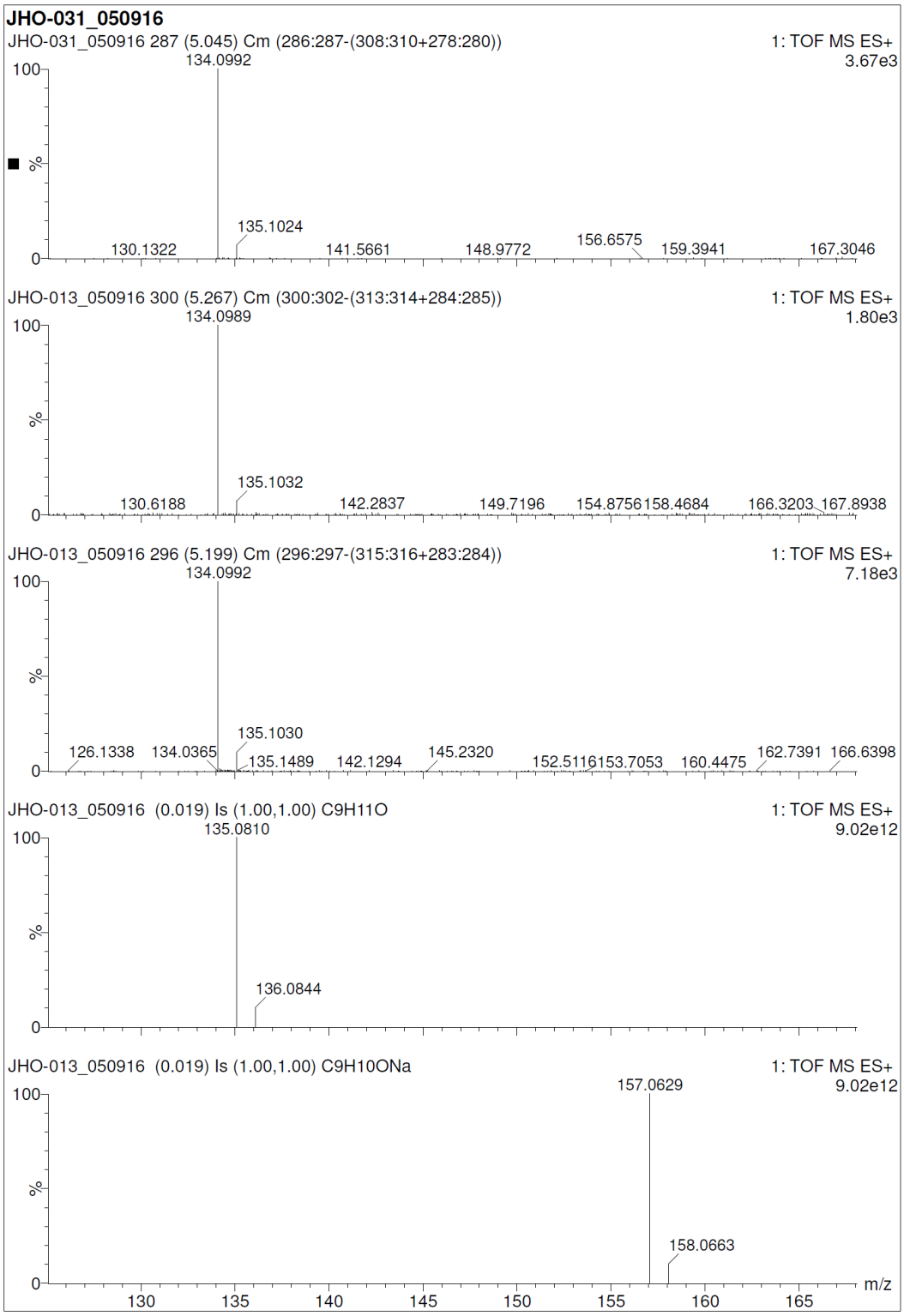
P2P 5, find mass MH TIC



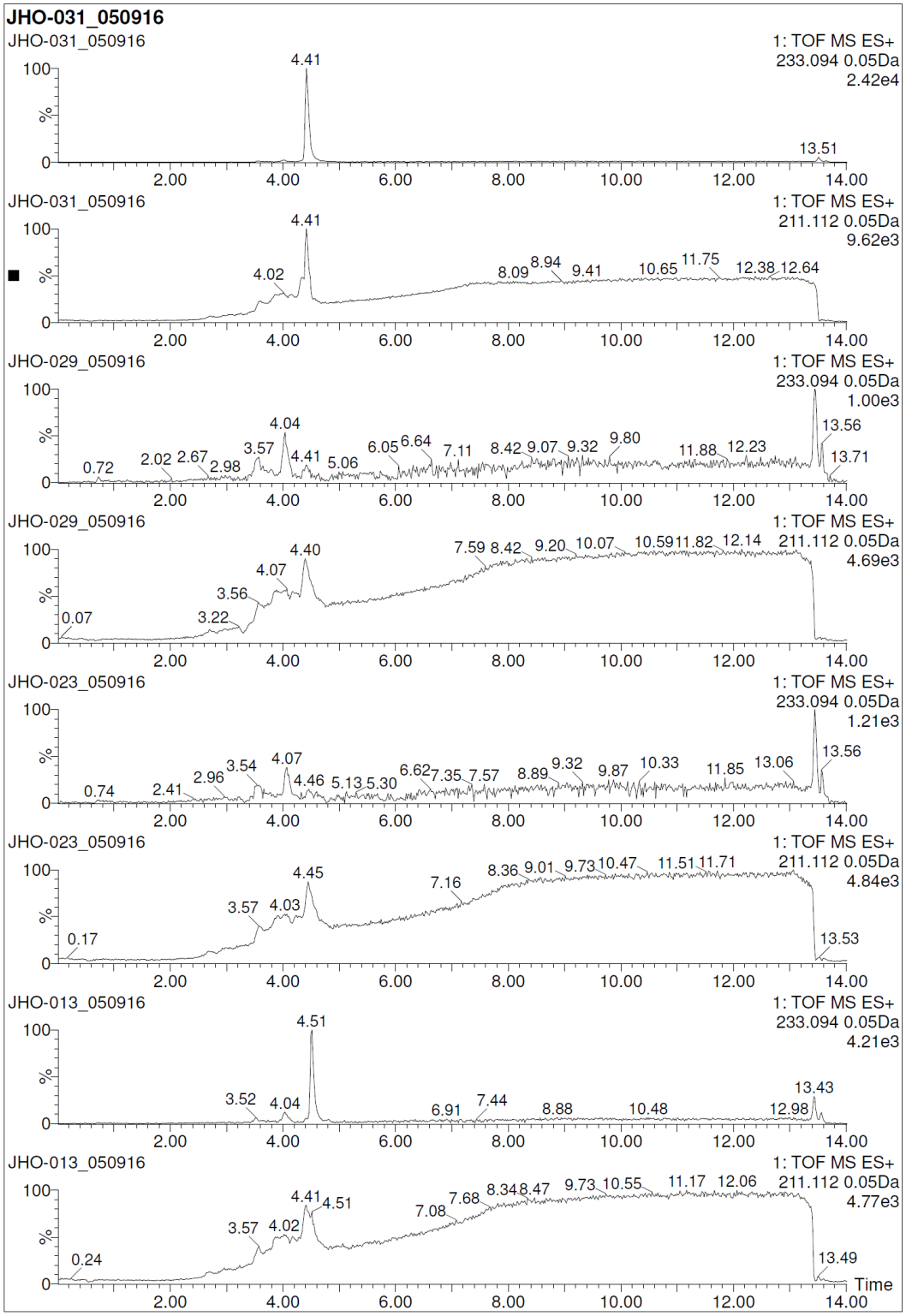
P2P 5, find mass MNa TIC



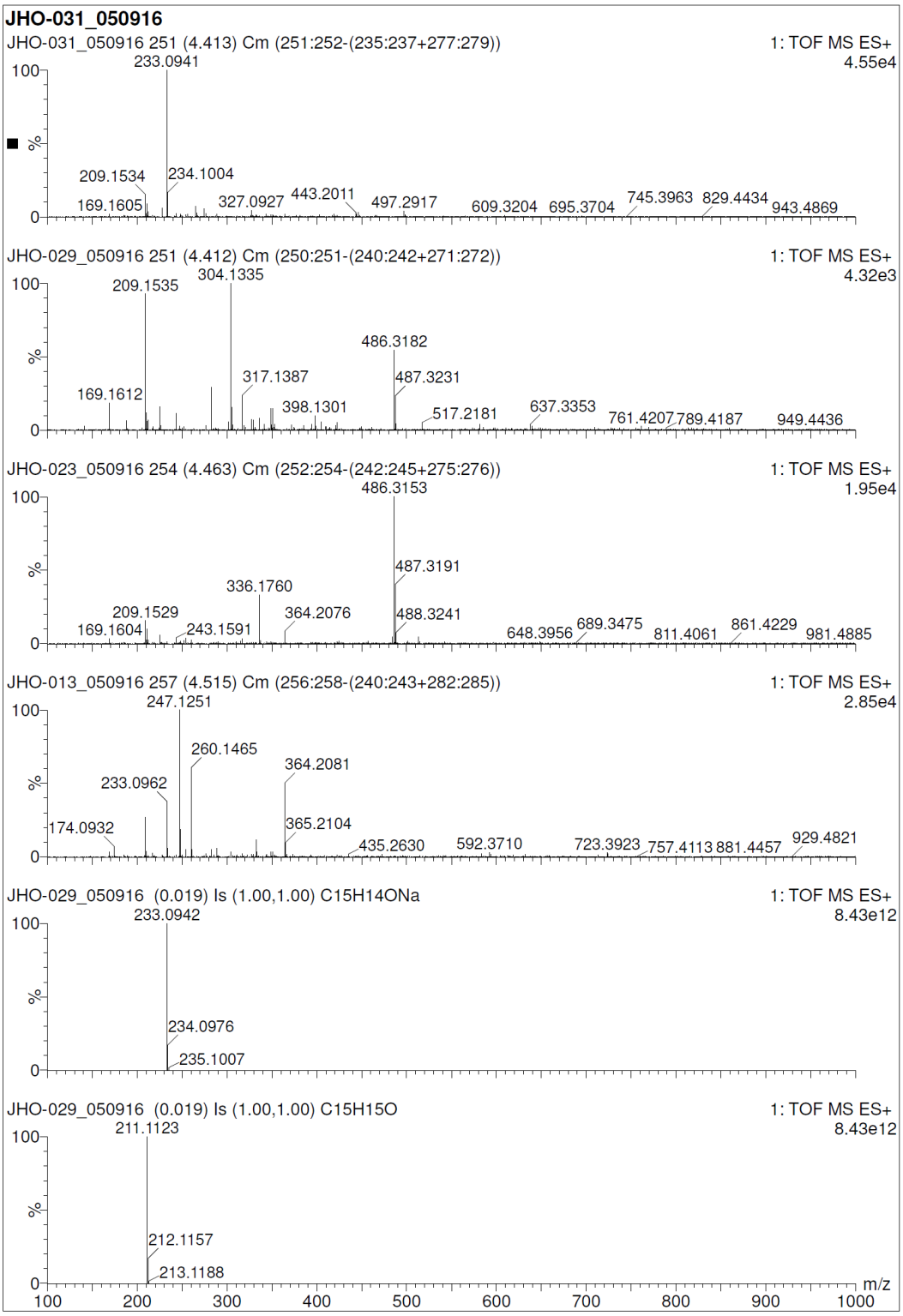
P2P 5 mass for retention time 5,20



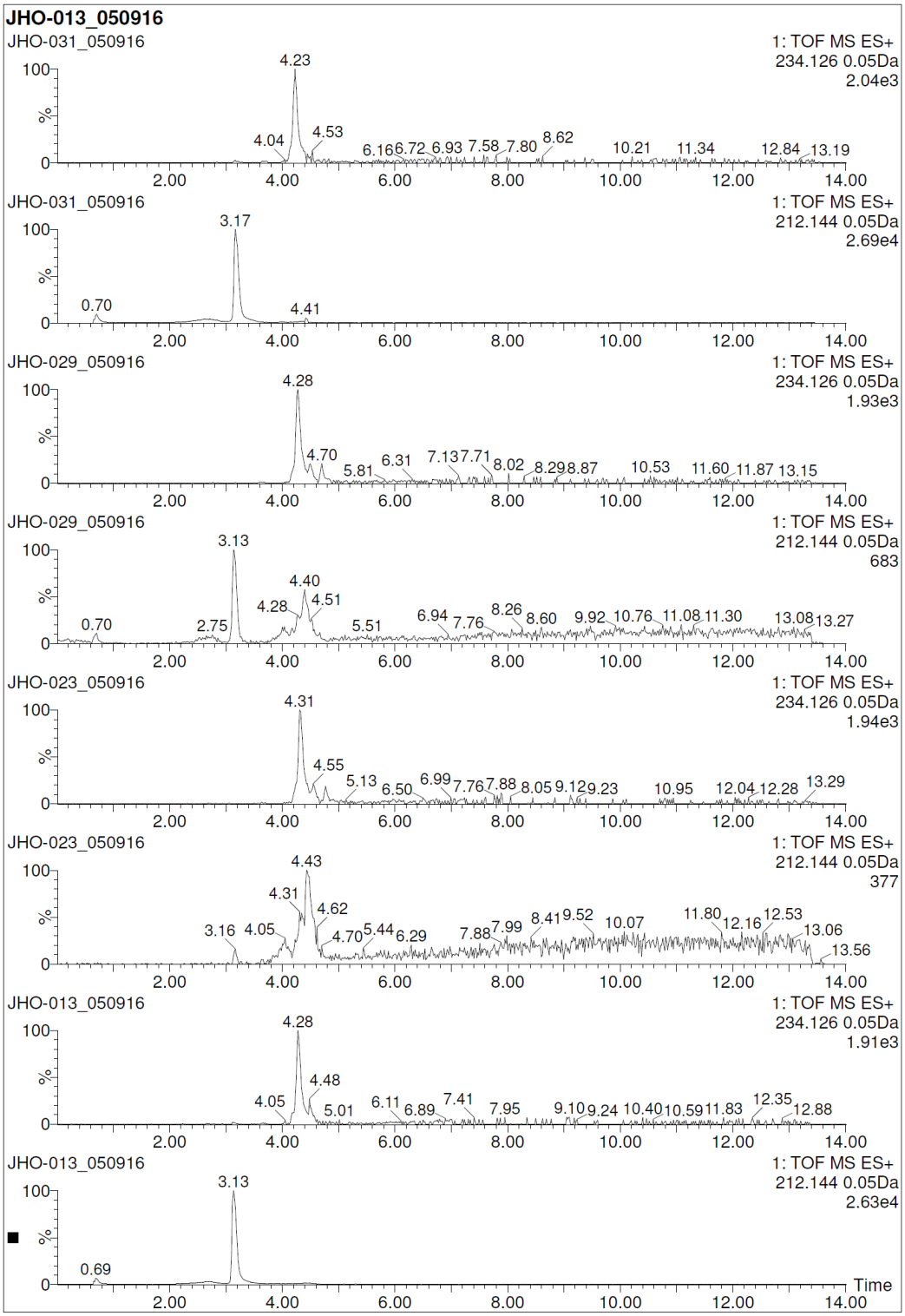
1,3-diphenyl-2-aminopropanone 6, find mass NH MNa TIC



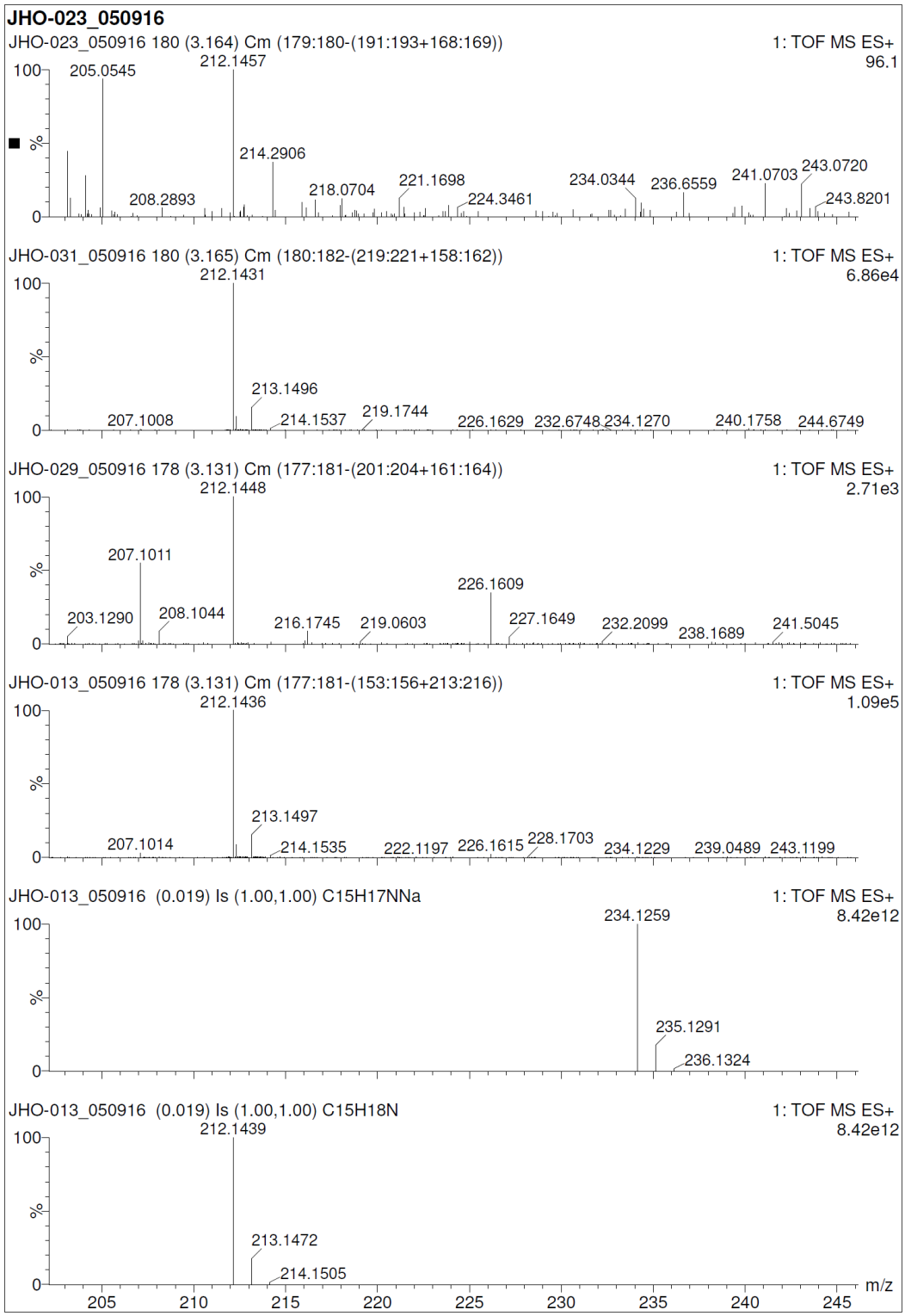
1,3-diphenyl-2-aminopropanone 6, mass for retention time 4,41-4,51



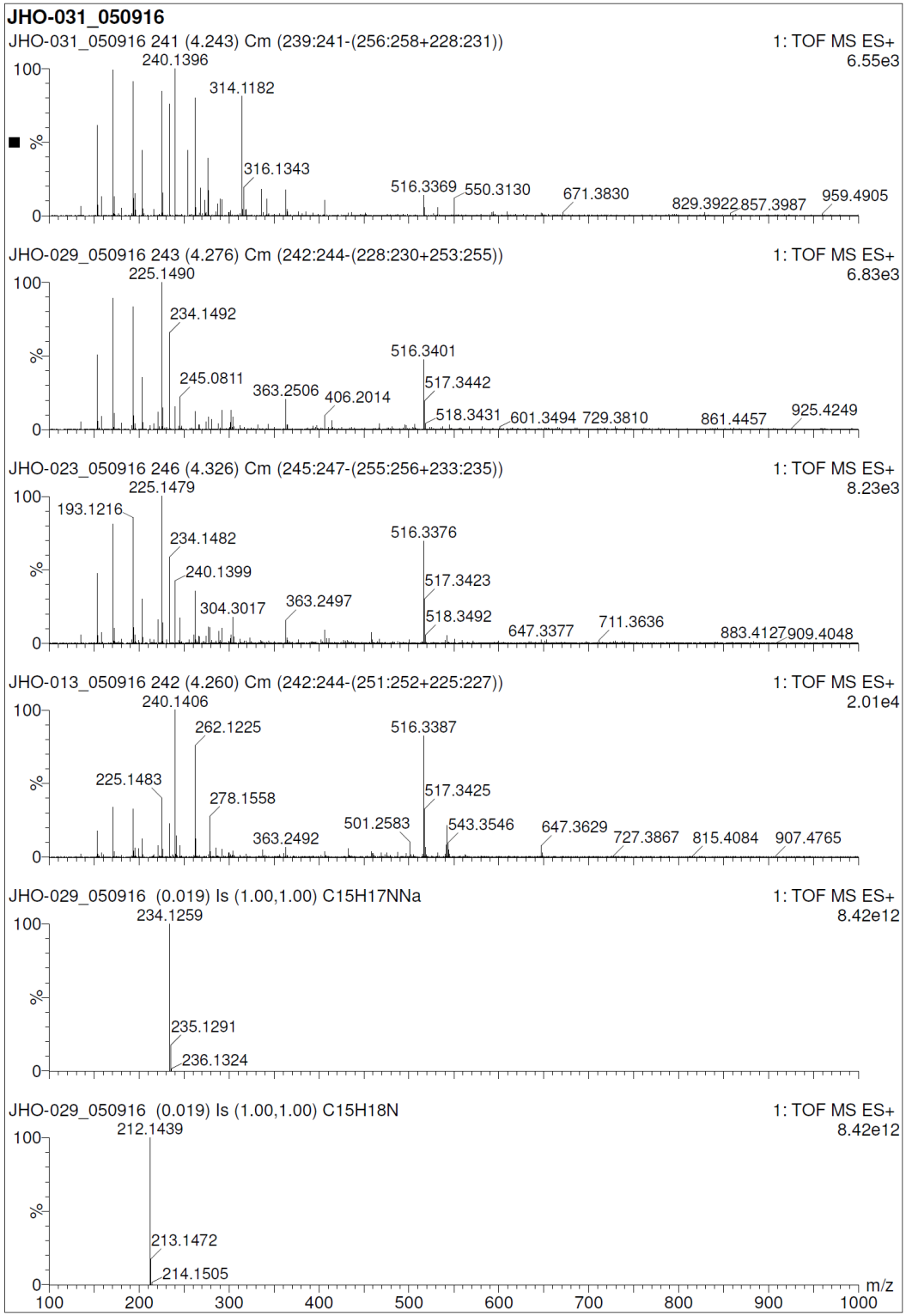
1,3-diphenyl-2-aminopropane 3, find mass MH MNa TIC



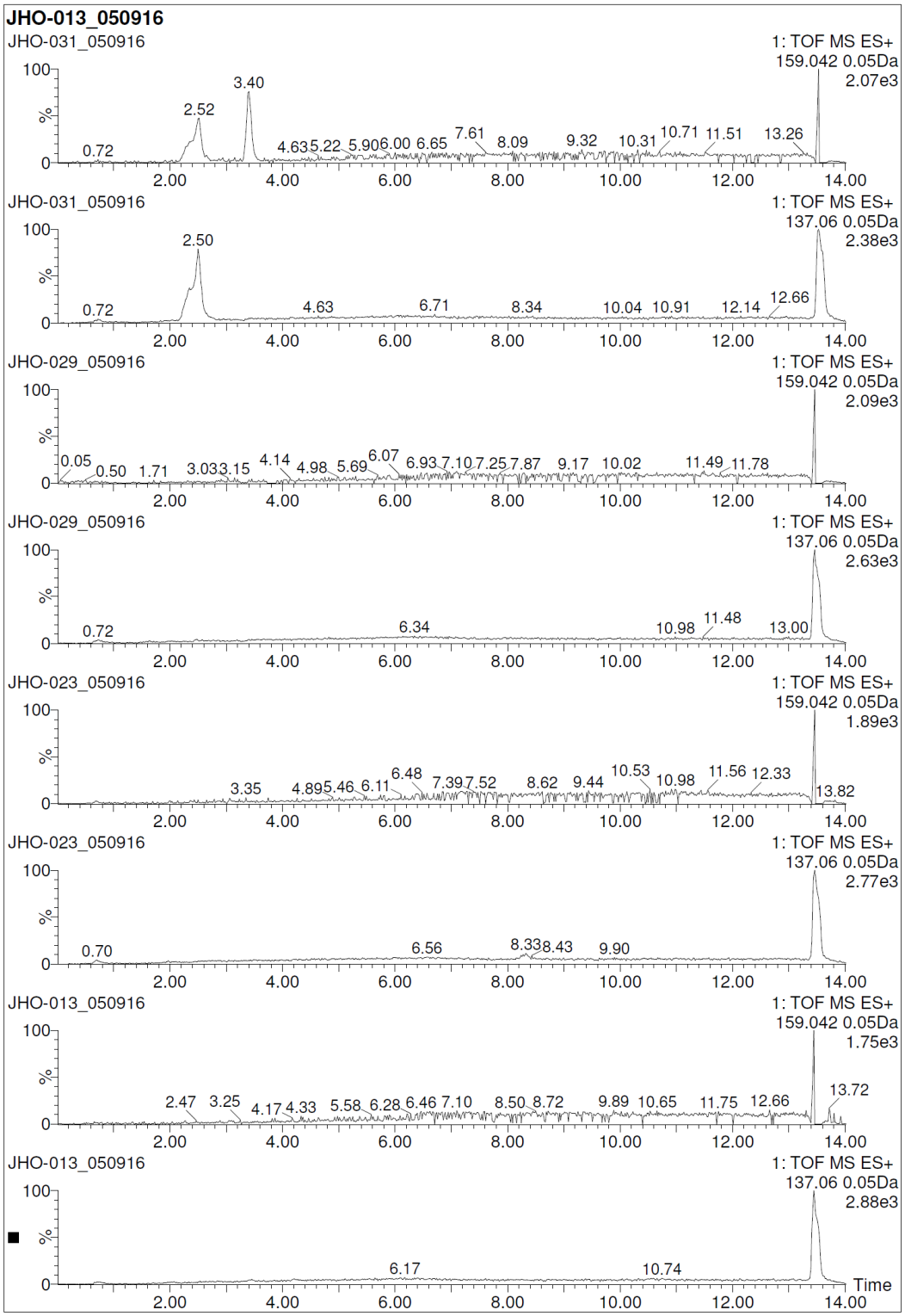
1,3-diphenyl-2-aminopropane 3, mass for retention time 3,13



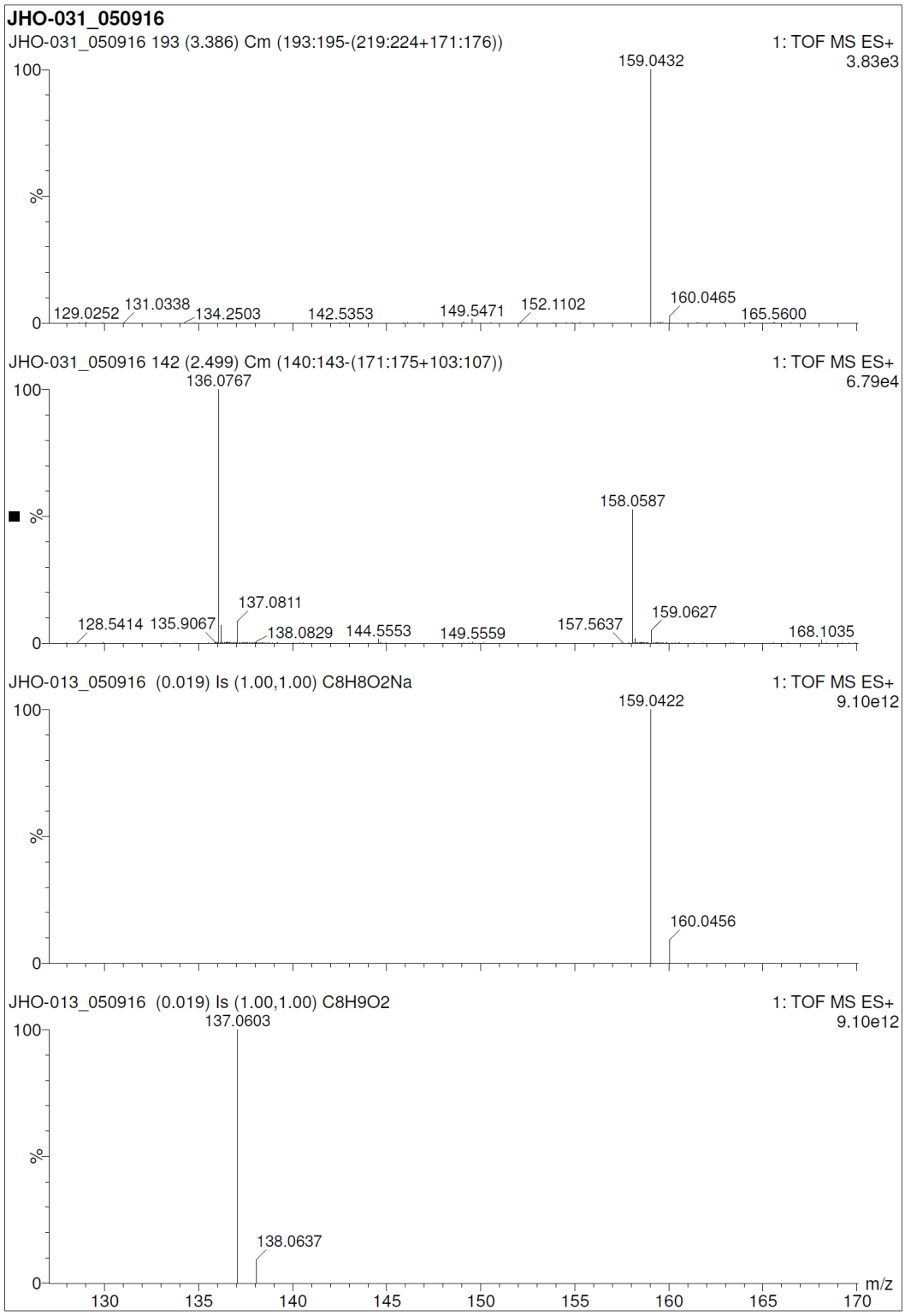
1,3-diphenyl-2-aminopropane 3, mass for retention time 4,28



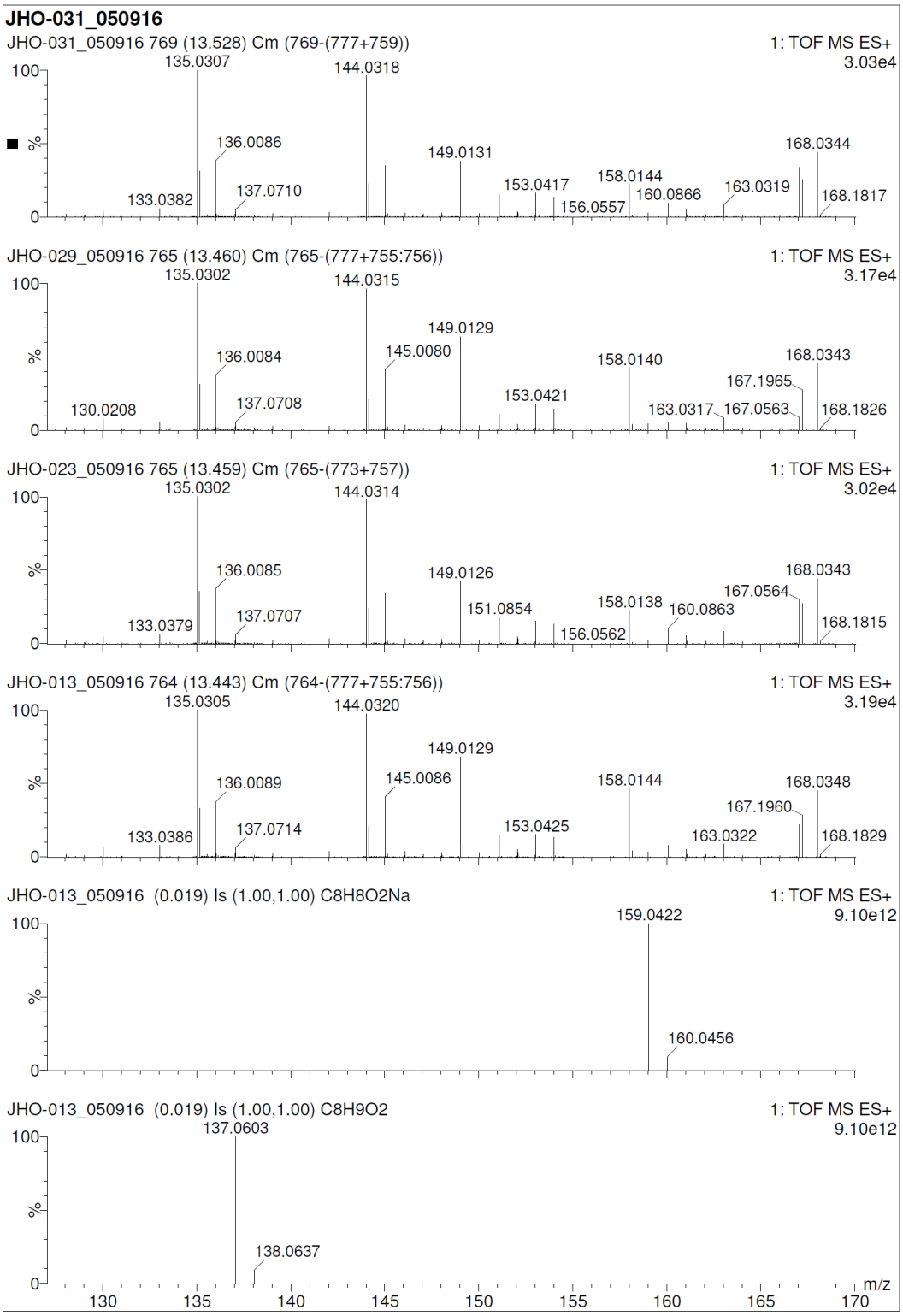
Phenylacetic acid 7, find mass MH MNa TIC



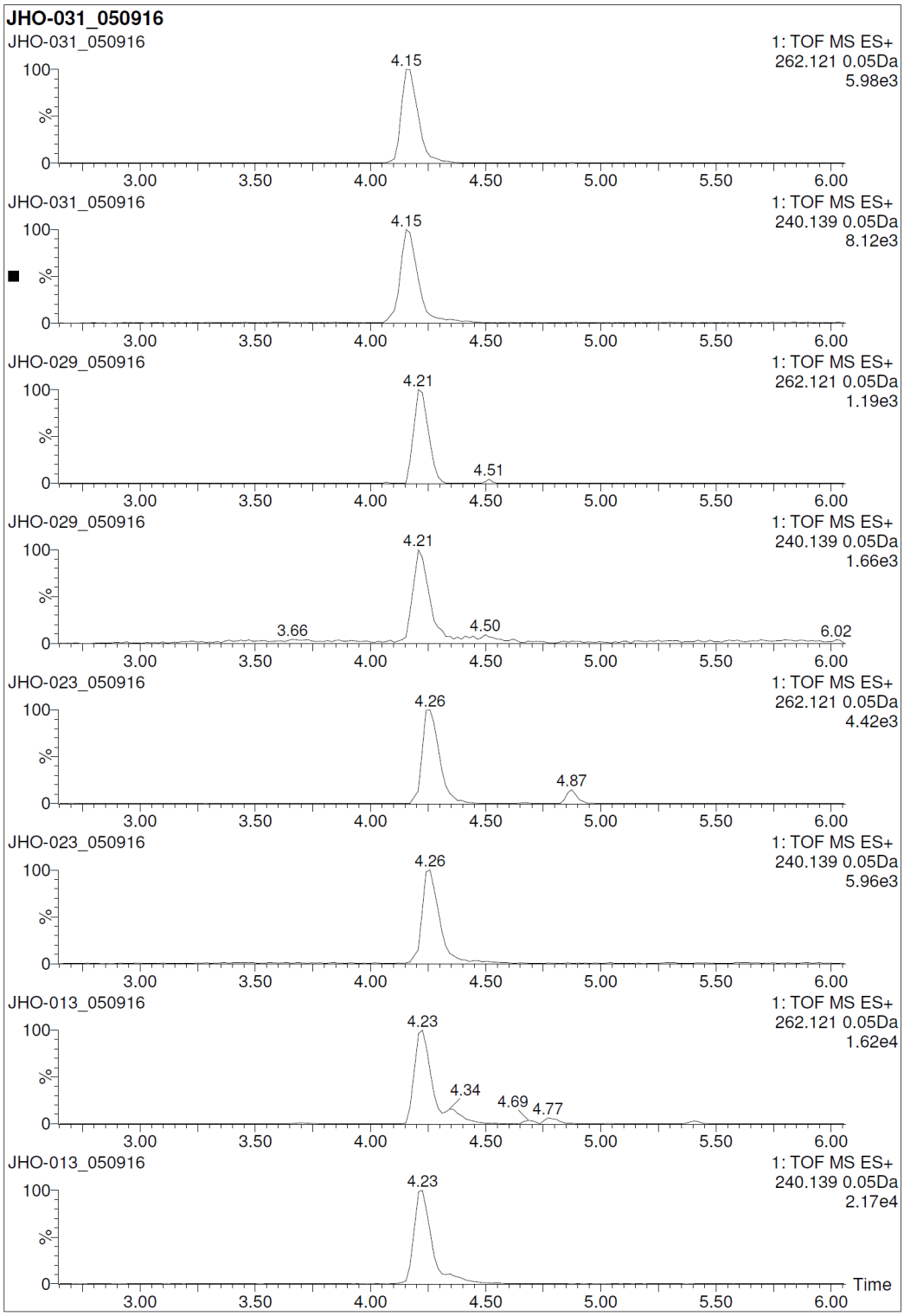
Phenylacetic acid 7, mass for retention time 2,50 and 3,40



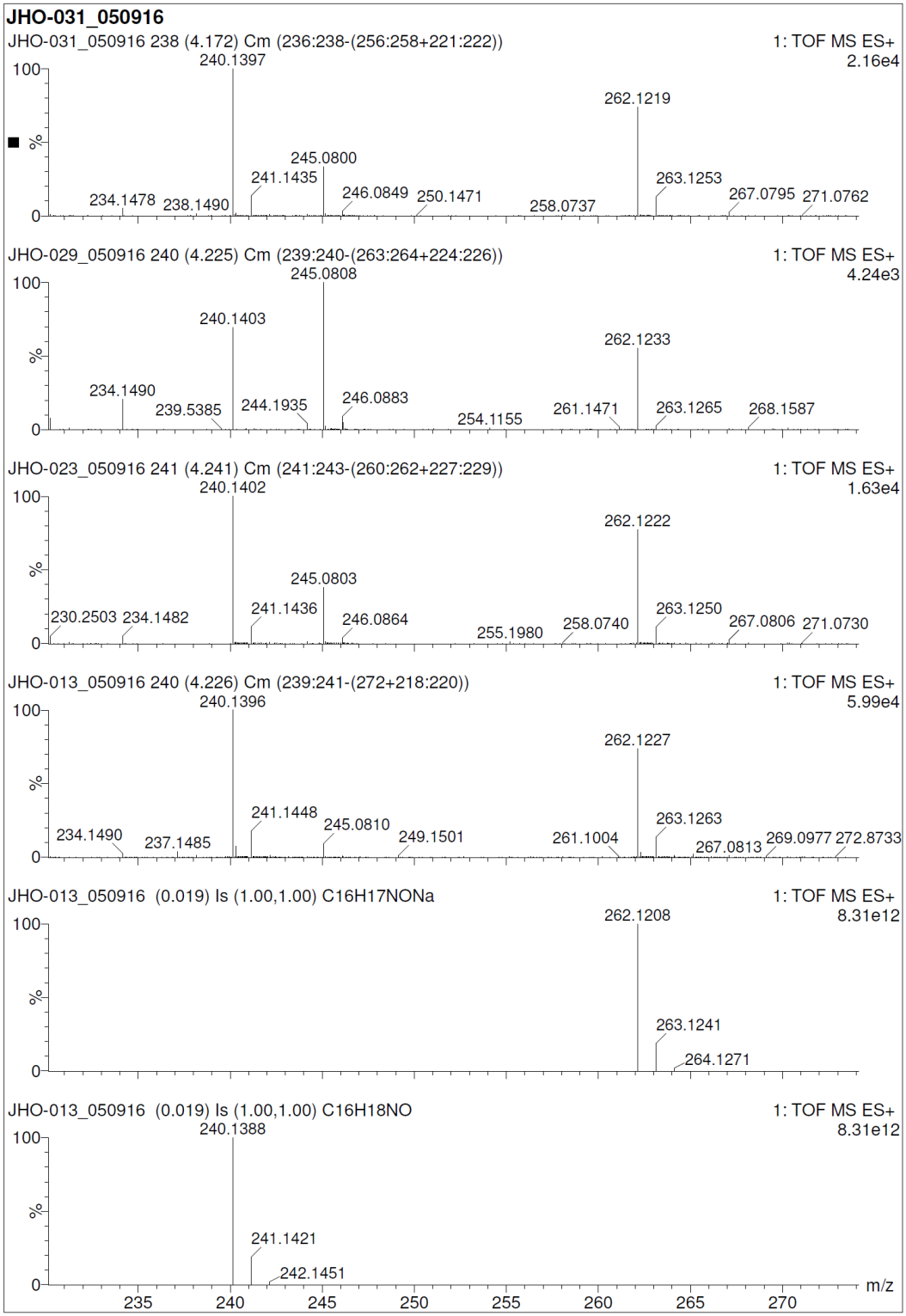
Phenylacetic acid 7, mass for retention time 13,46



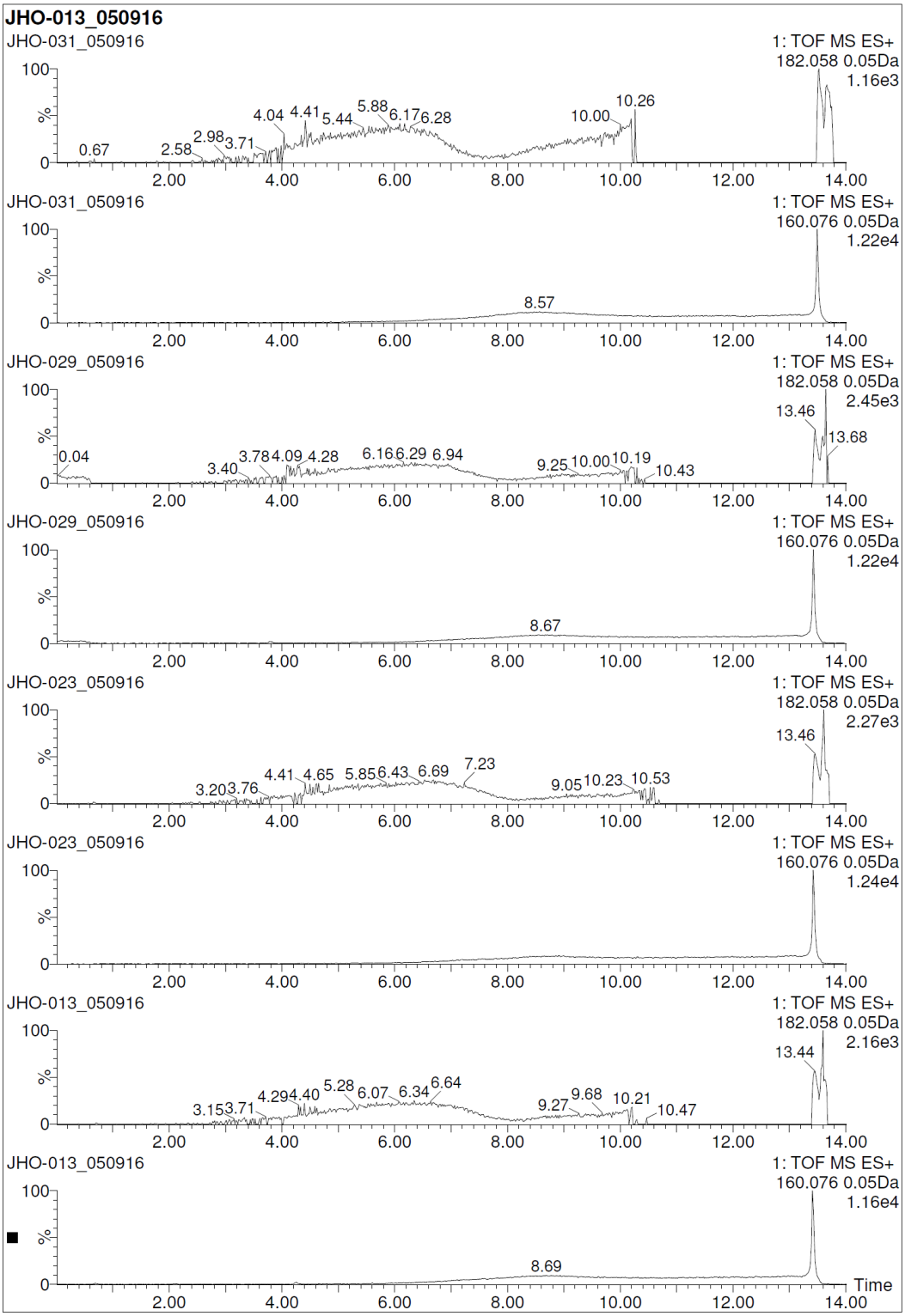
Benzylphenylethylamineformamide 4 find mass MH MNa TIC



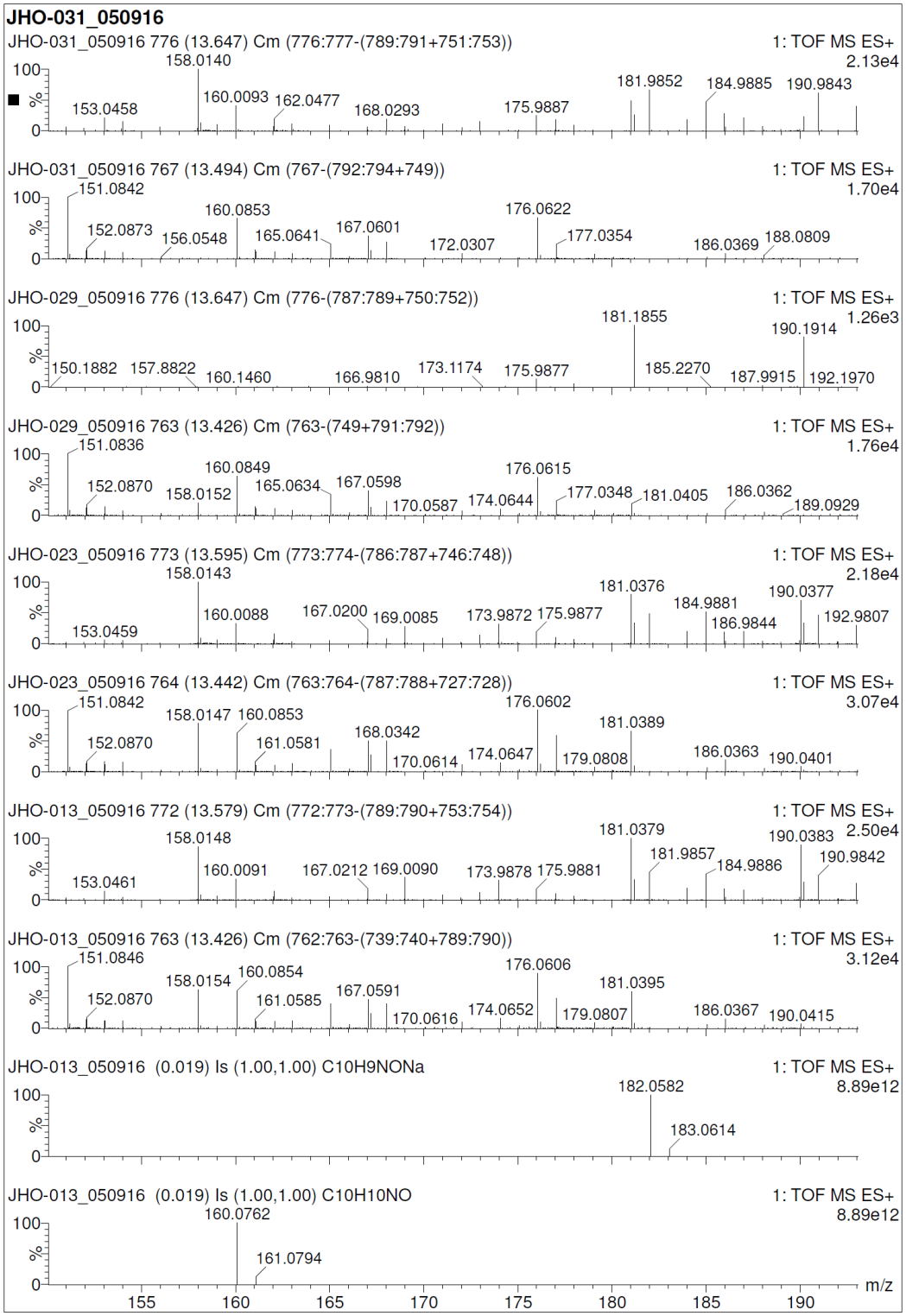
Benzylphenylethylamineformamide 4 mass at retention time 4,23



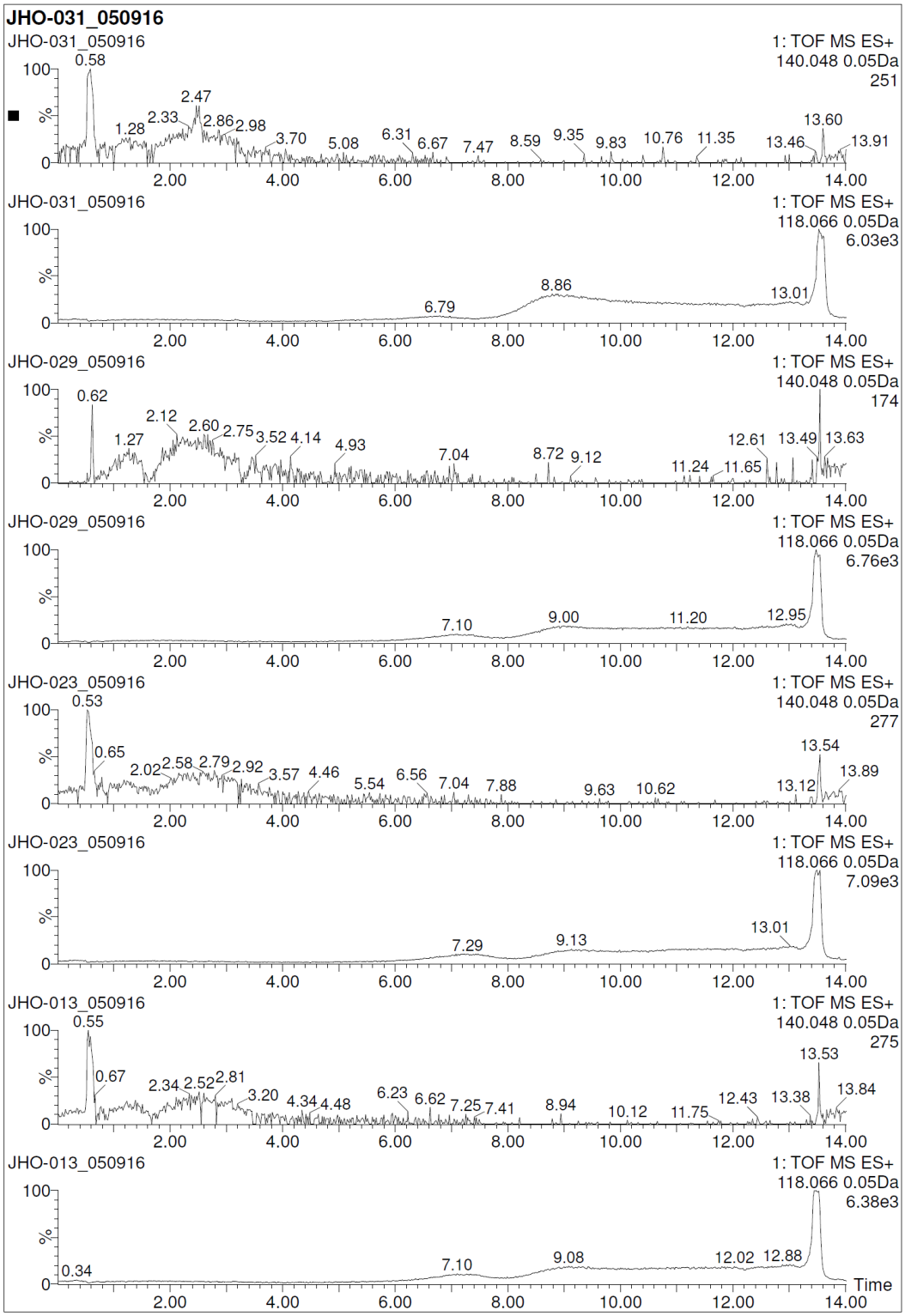
APAAN 10 find mass MH MNa TIC



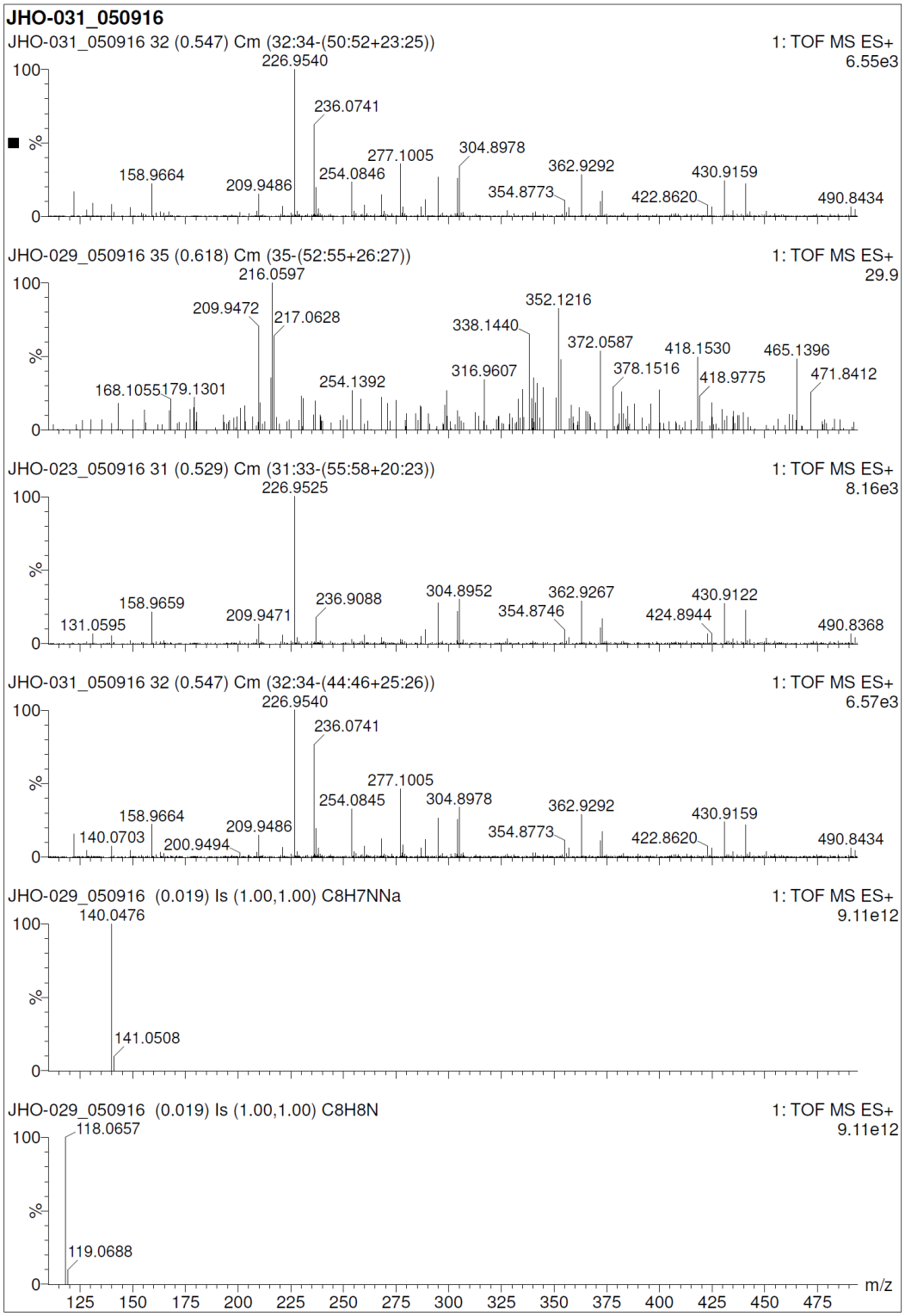
APAAN 10 find mass at retention time 13,46 and 13,60



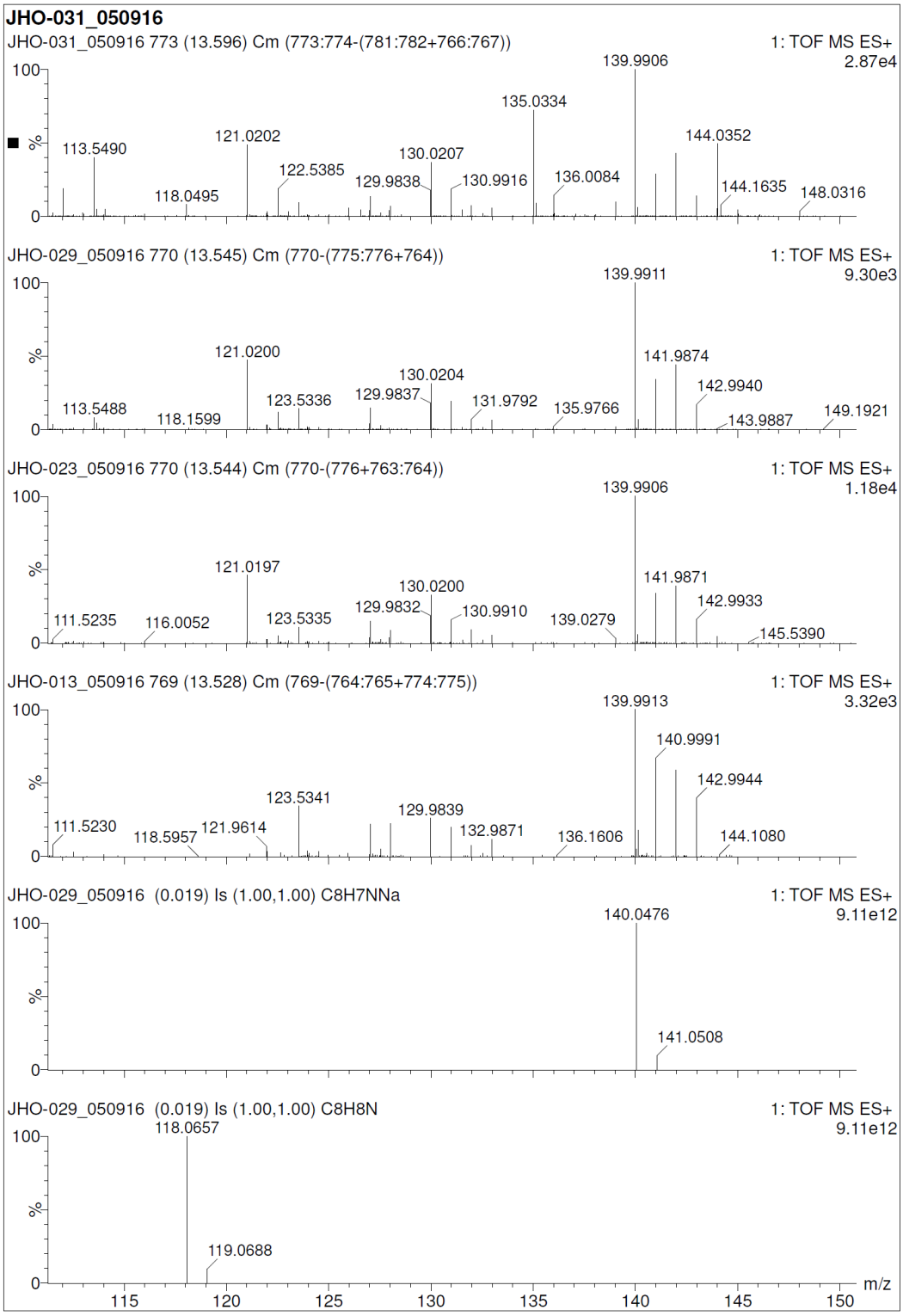
Benzyl Cyanide 17 find mass MH MNa TIC



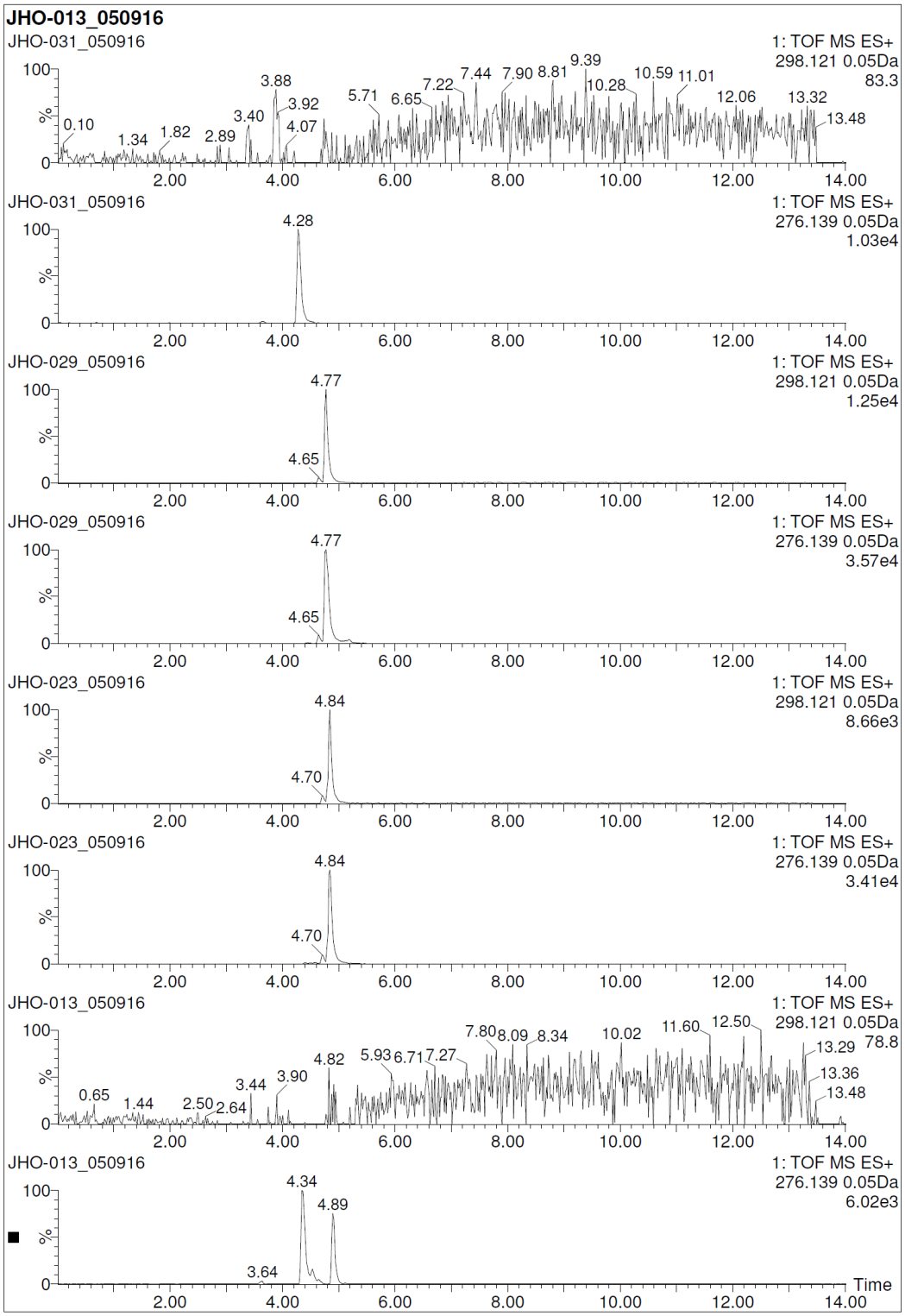
Benzyl Cyanide 17 mass at retention time 0,53



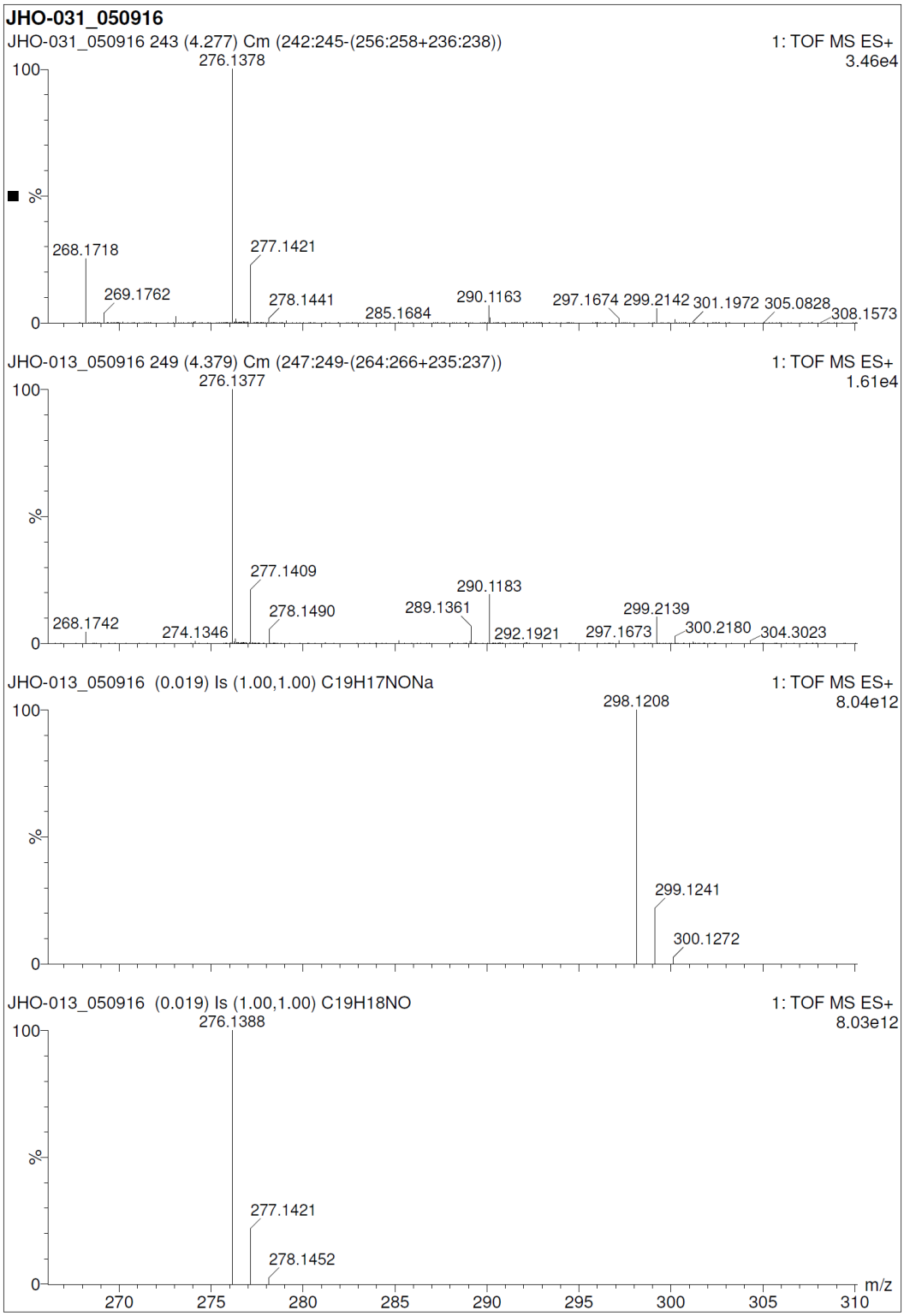
Benzyl Cyanide 17 mass at retention time 13,53



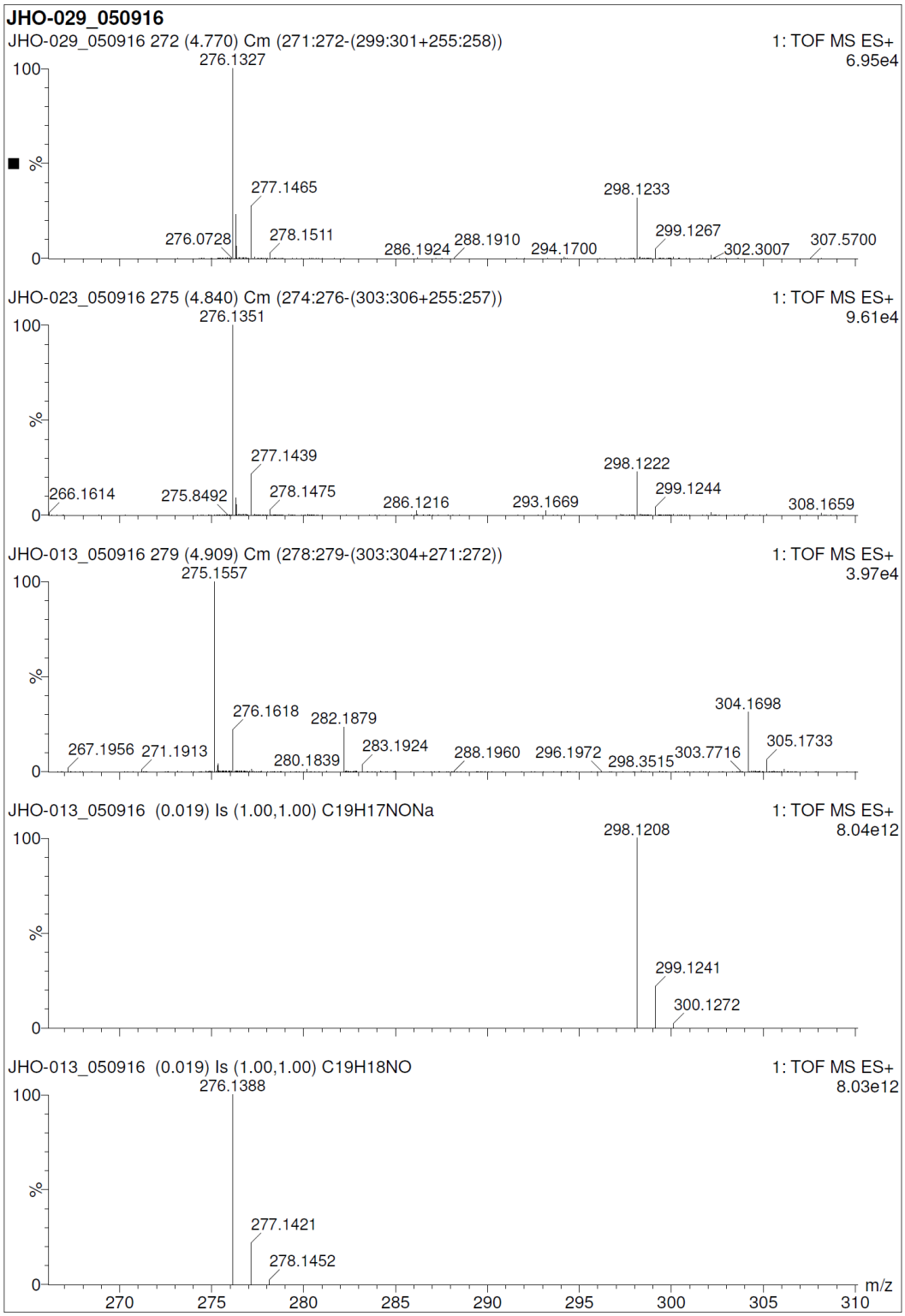
4,6-dimethyl-3,5-diphenylpyridine-2-one 9 find mass MH Ma TIC



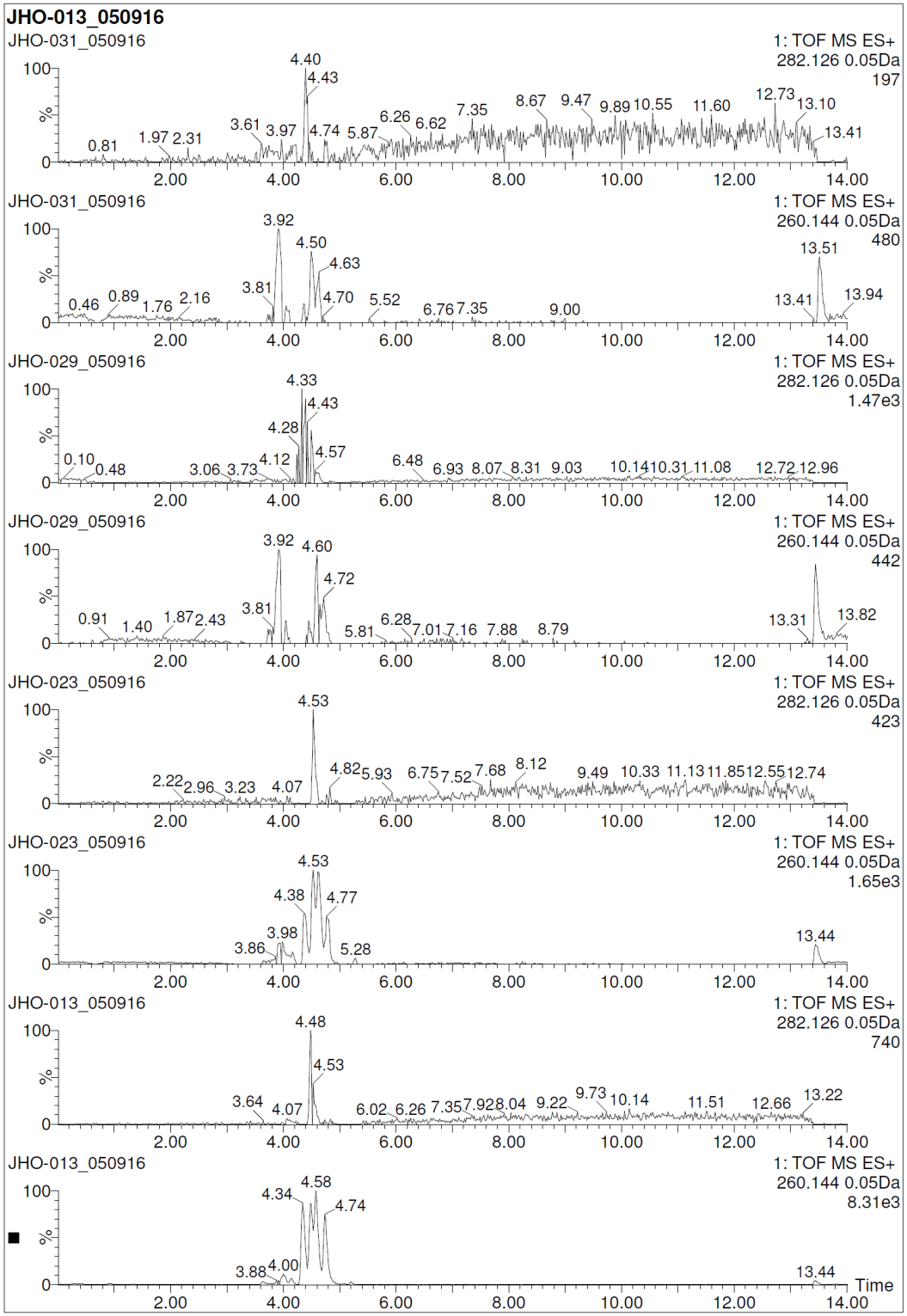
4,6-dimethyl-3,5-diphenylpyridine-2-one mass at retention time 4,28 and 4,34



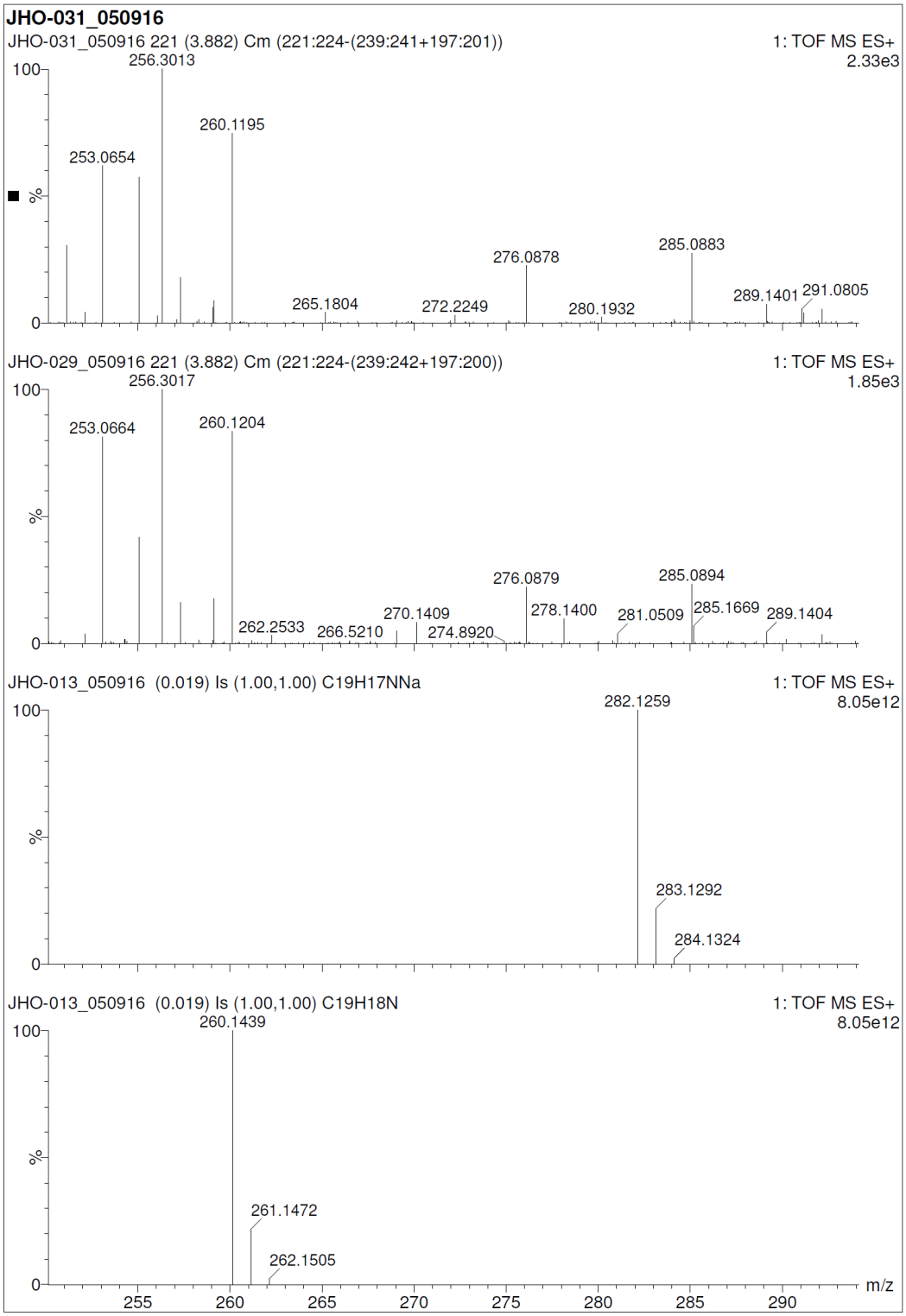
4,6-dimethyl-3,5-diphenylpyridine-2-one mass at retention time 4,77 and 4,84



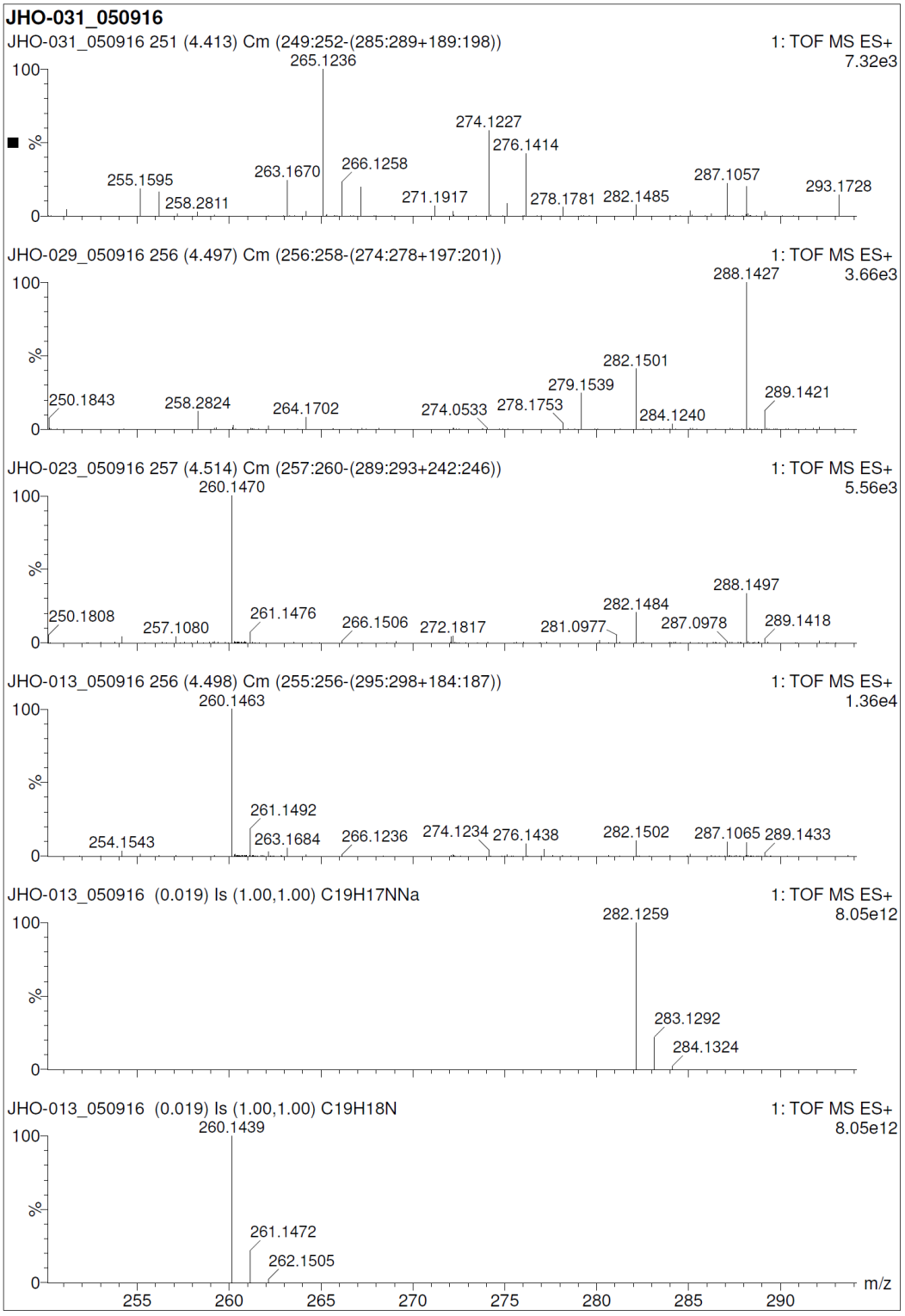
2,6-dimethyl-3,5-diphenylpyridine 12b or 2,4-dimethyl-3,5-diphenylpyridine 12a find mass MH MNa TIC



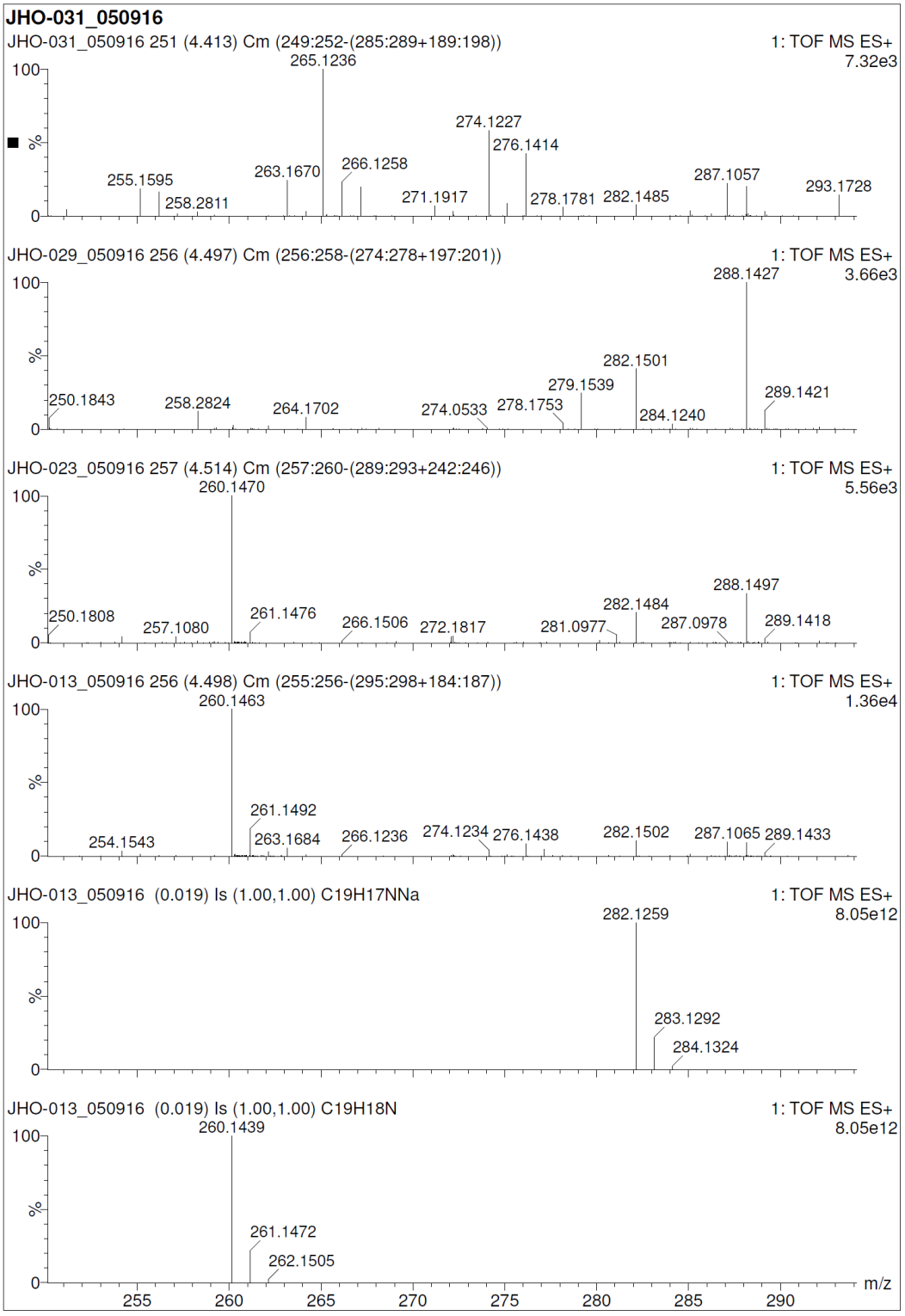
2,6-dimethyl-3,5-diphenylpyridine 12b or 2,4-dimethyl-3,5-diphenylpyridine 12a mass at 3,92



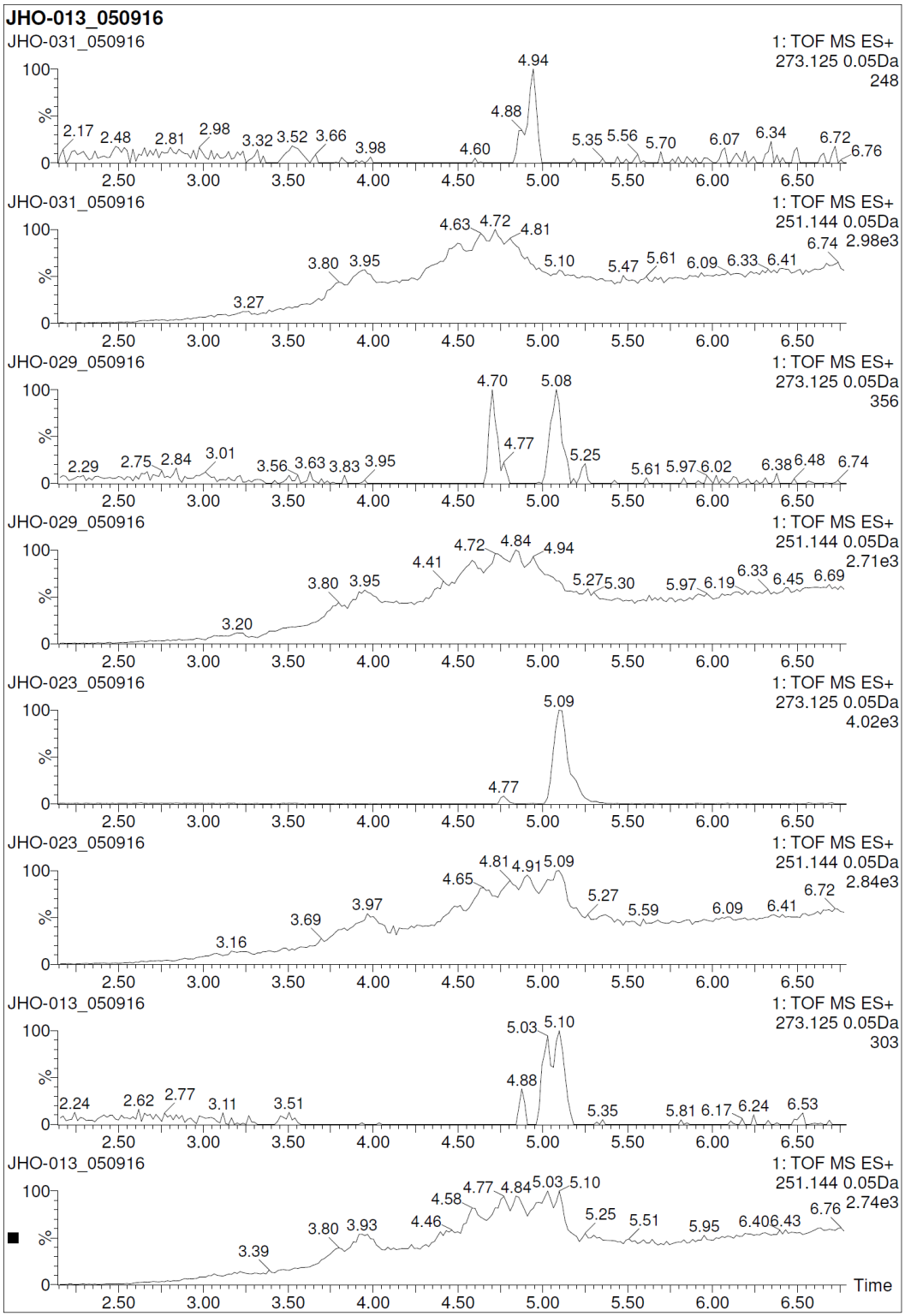
2,6-dimethyl-3,5-diphenylpyridine 12b or 2,4-dimethyl-3,5-diphenylpyridine 12a mass at 3,92



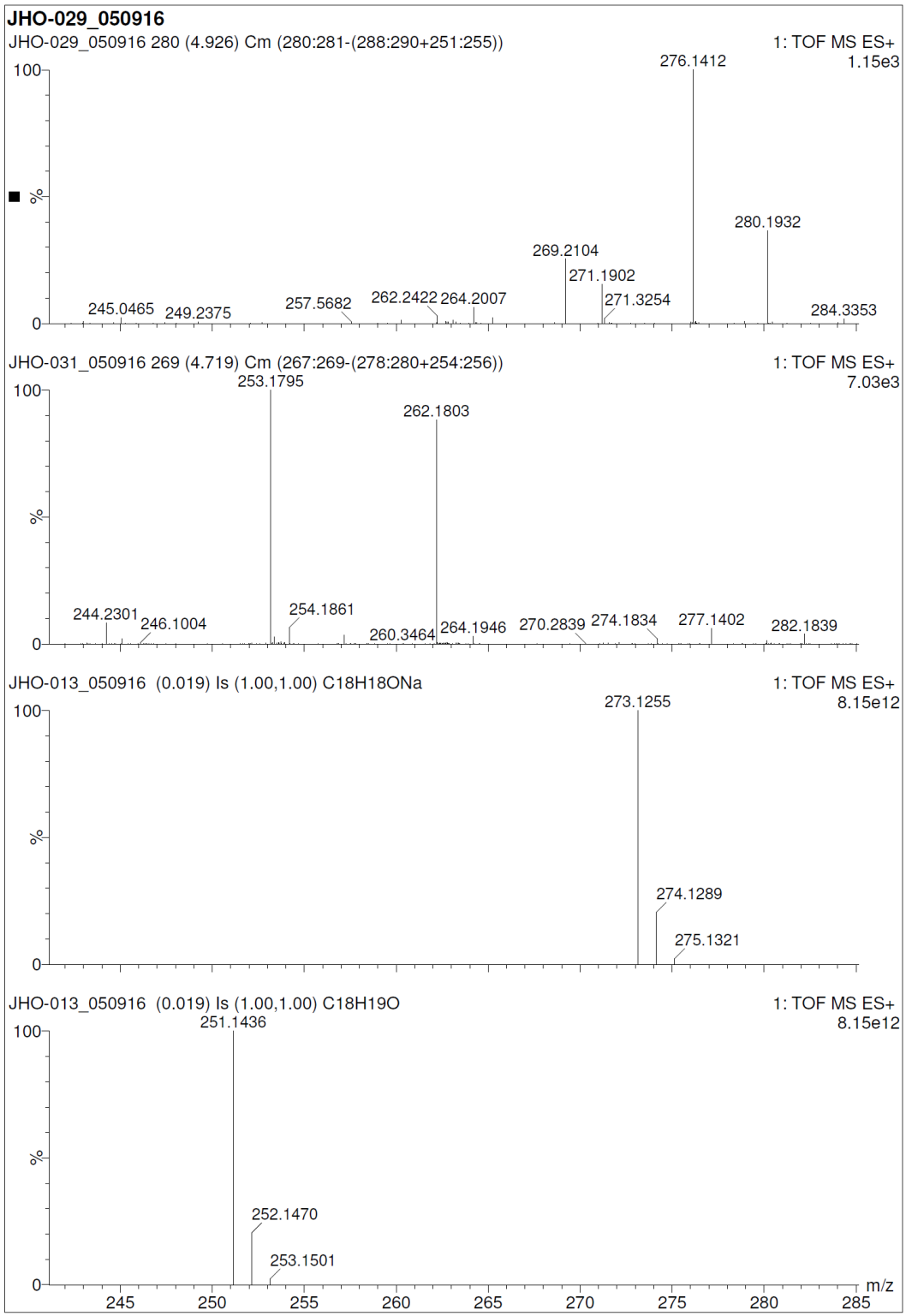
2,6-dimethyl-3,5-diphenylpyridine 12b or 2,4-dimethyl-3,5-diphenylpyridine 12a mass at 4,48



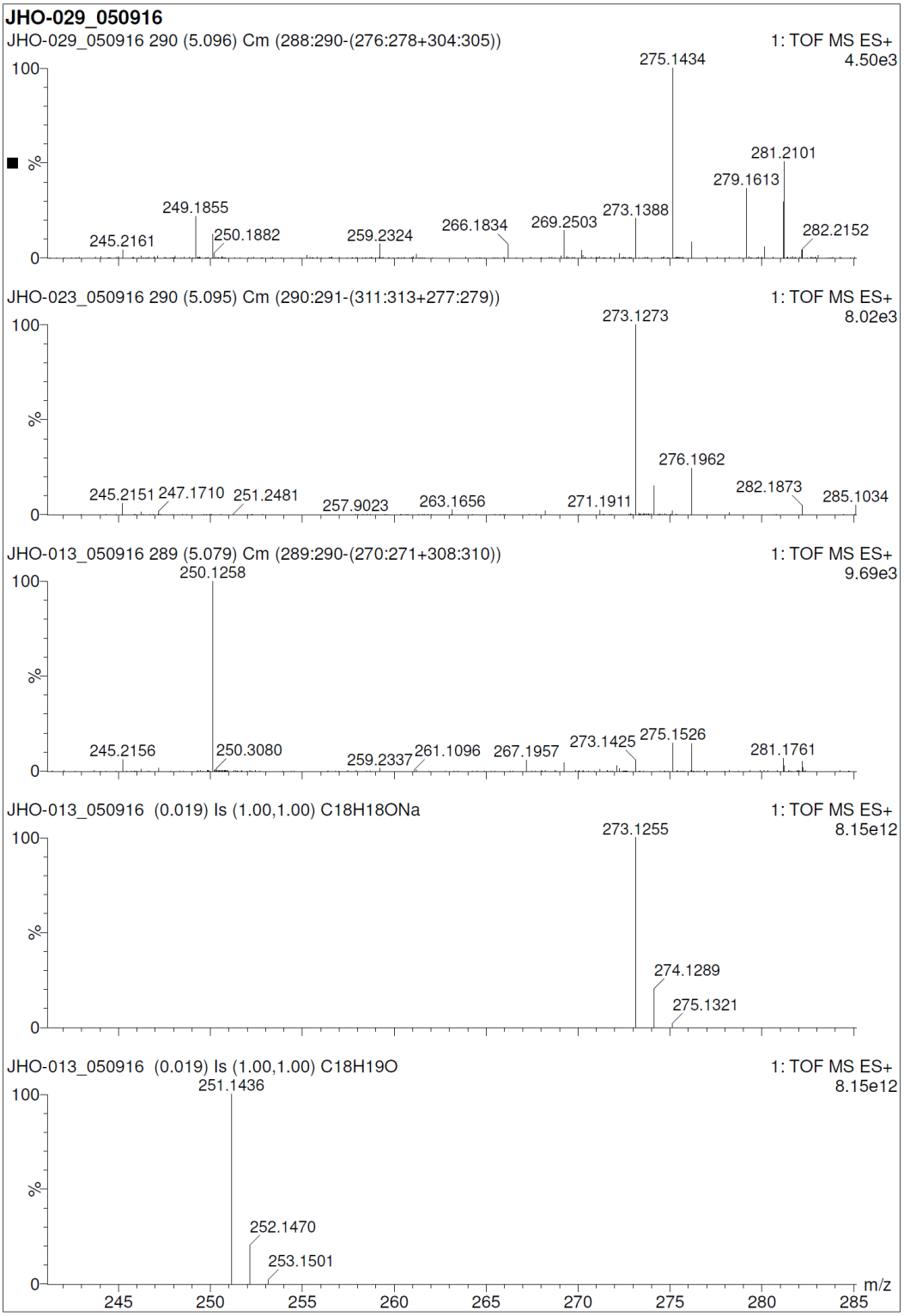
1,5-diphenyl-2-methyl-4-oxopent-1-ene 15 find mass MH MNa TIC



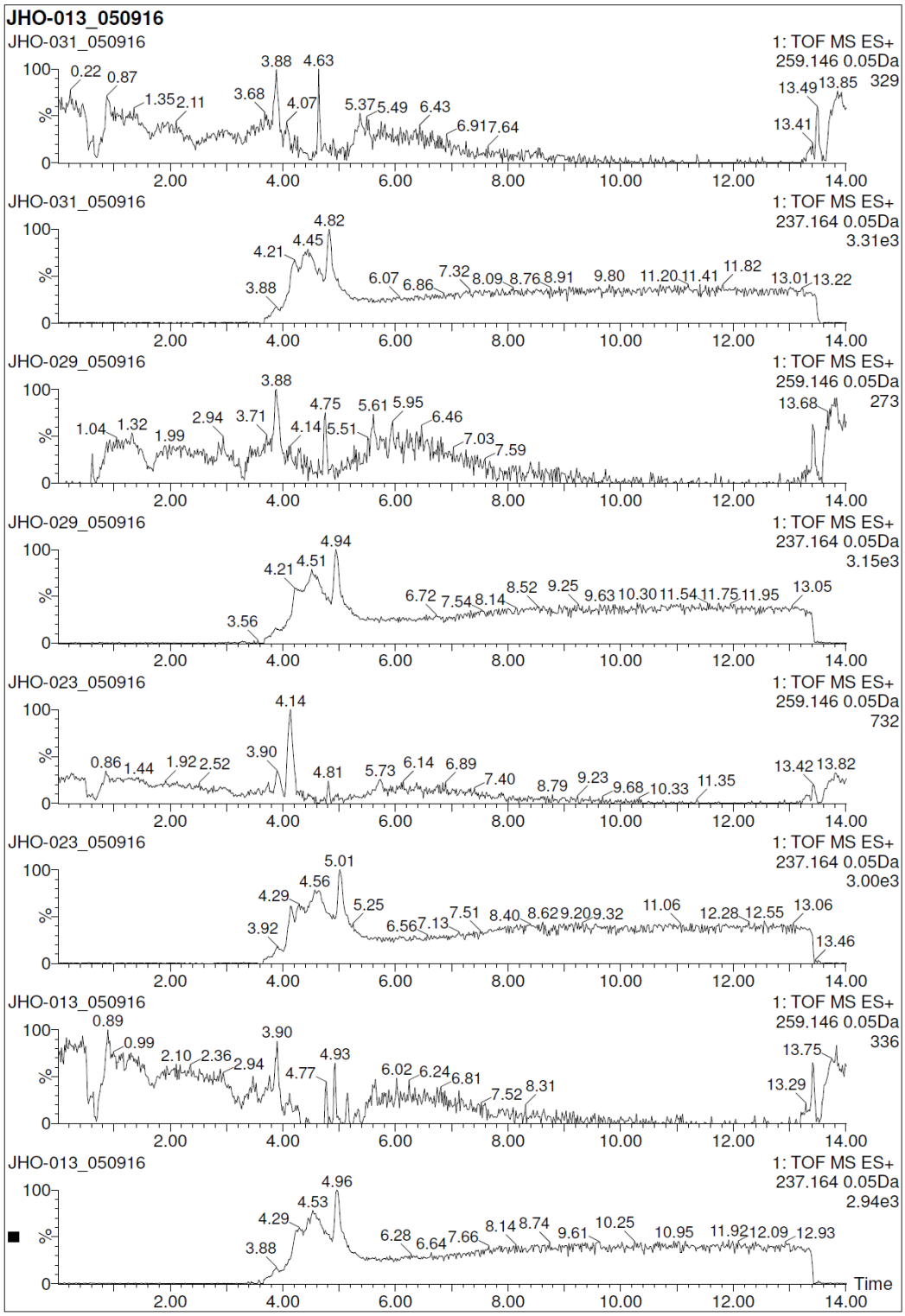
1,5-diphenyl-2-methyl-4-oxopent-1-ene 15 mass at retention time 4,70 and 4,94



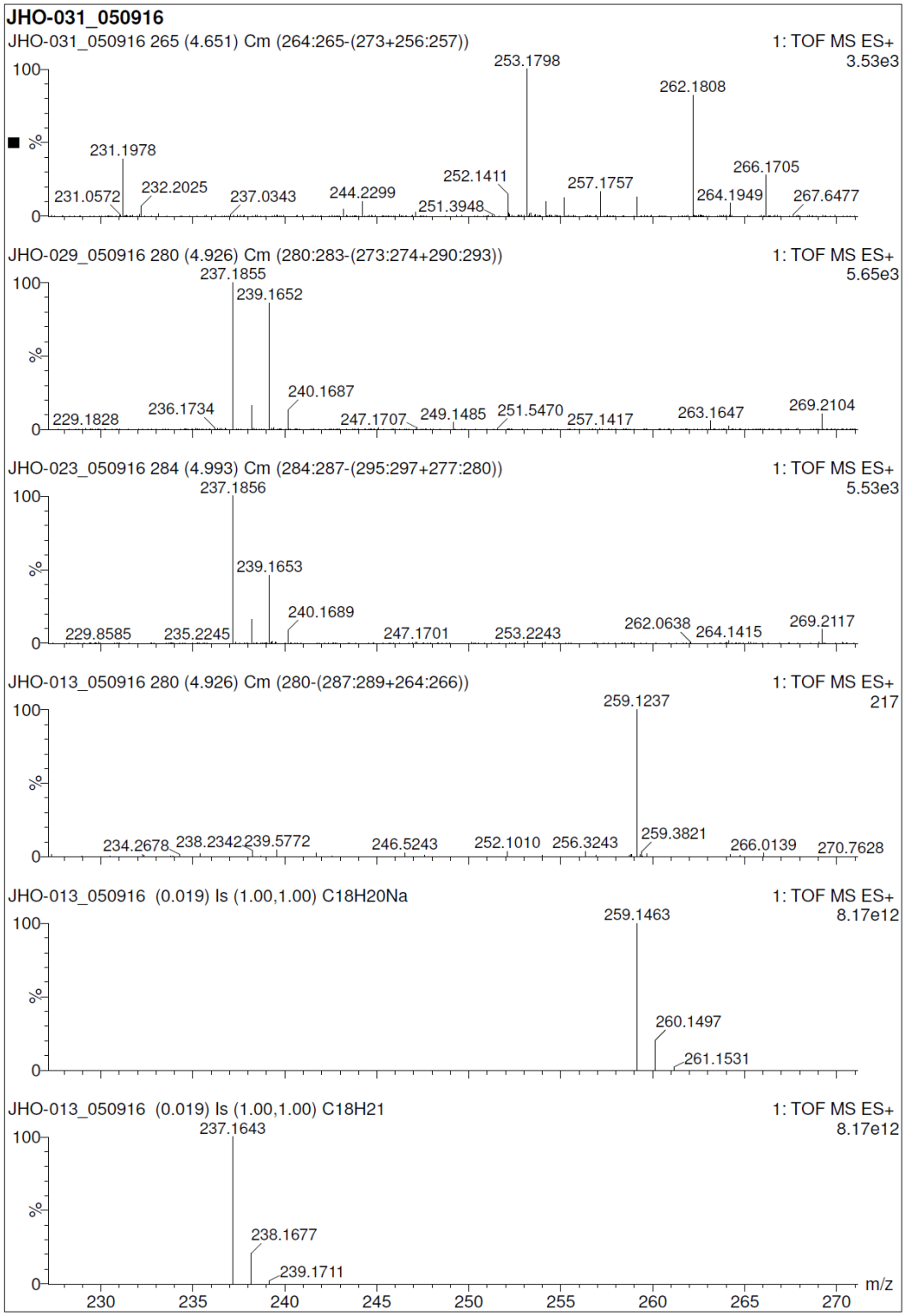
1,5-diphenyl-2-methyl-4-oxopent-1-ene 15 mass at retention time 5,10



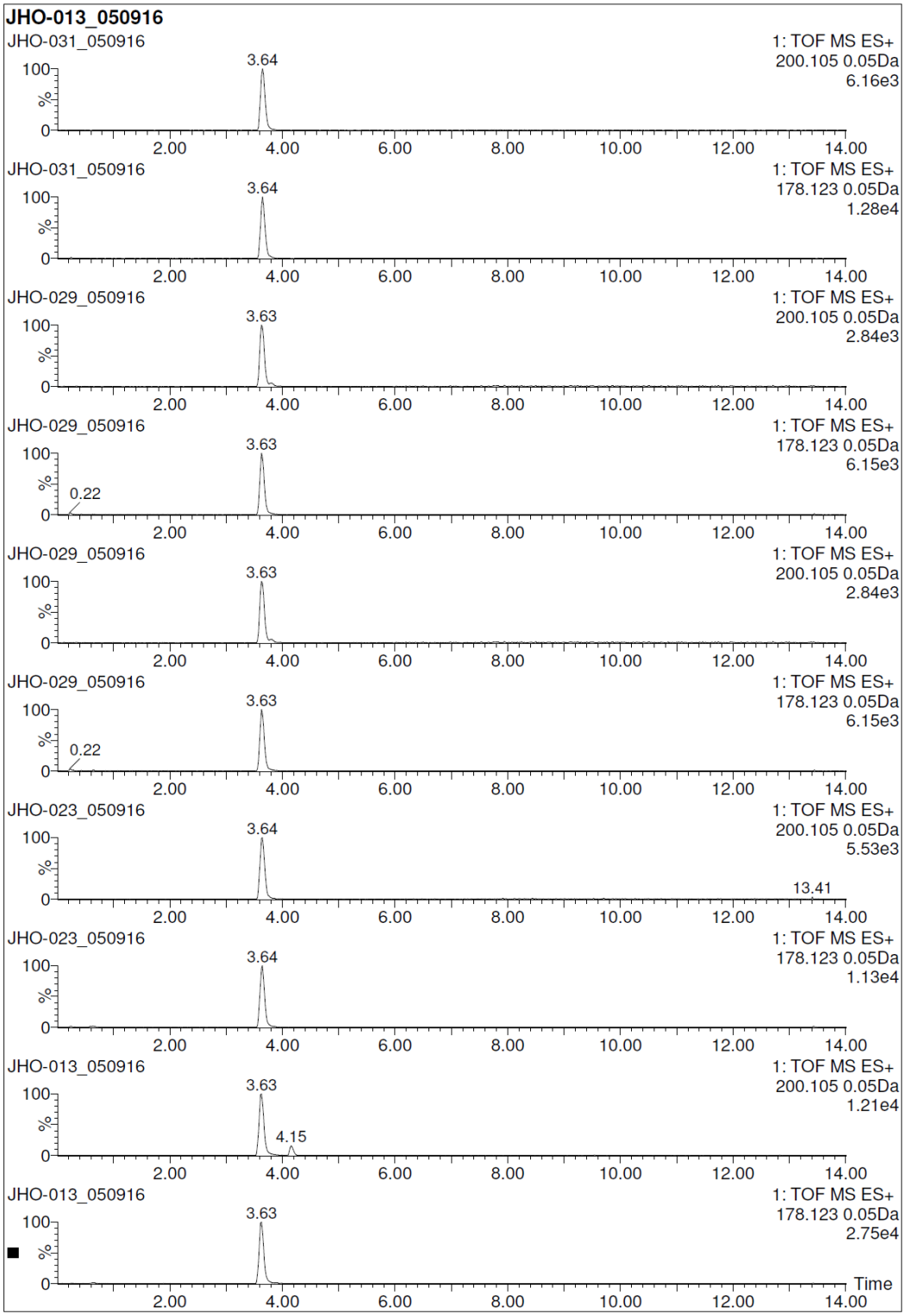
1,5-diphenyl-4-methylpent-1-ene 14 find mass MH MNa TIC



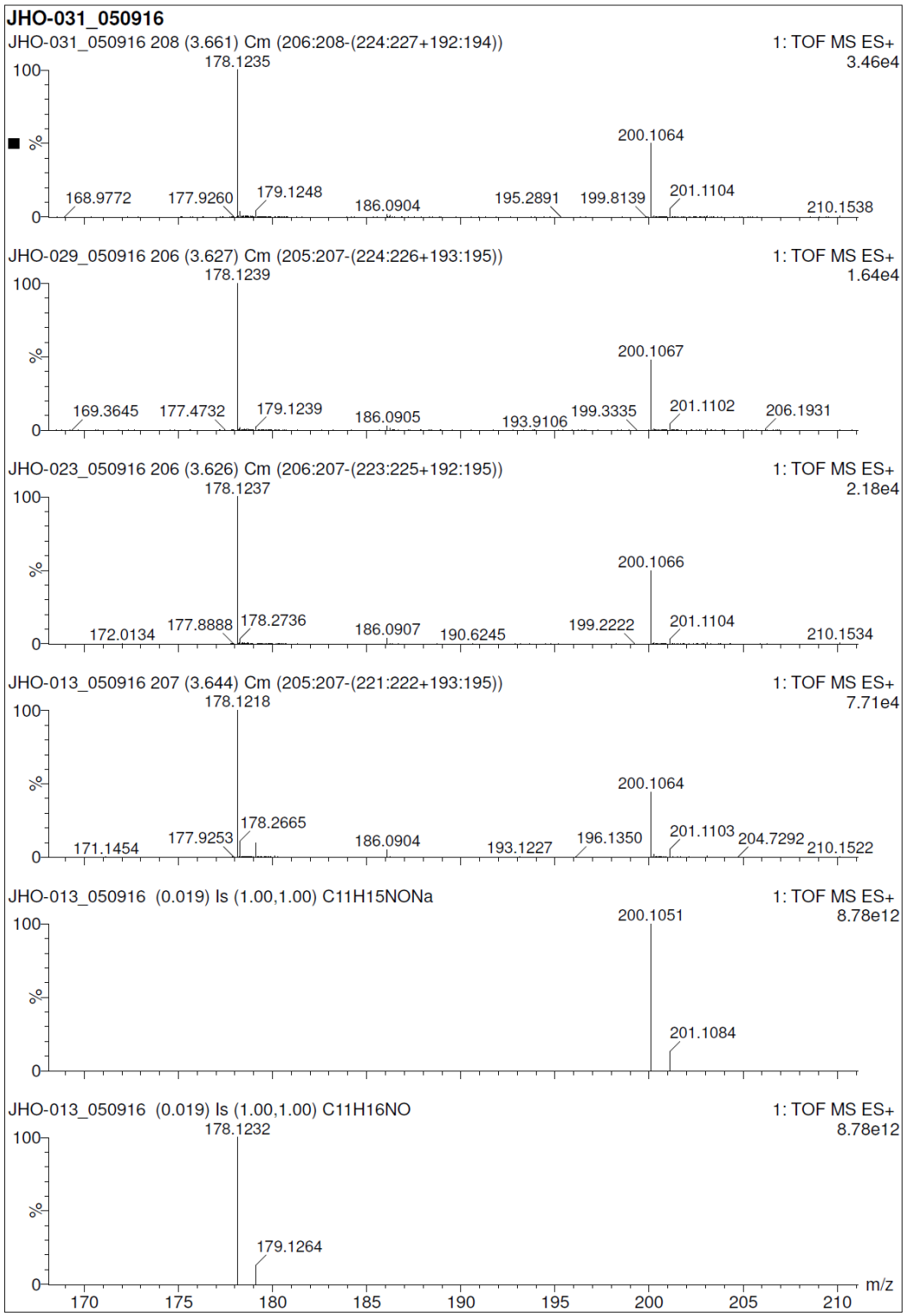
1,5-diphenyl-4-methylpent-1-ene 14 mass at retention time 4,63 – 5,10



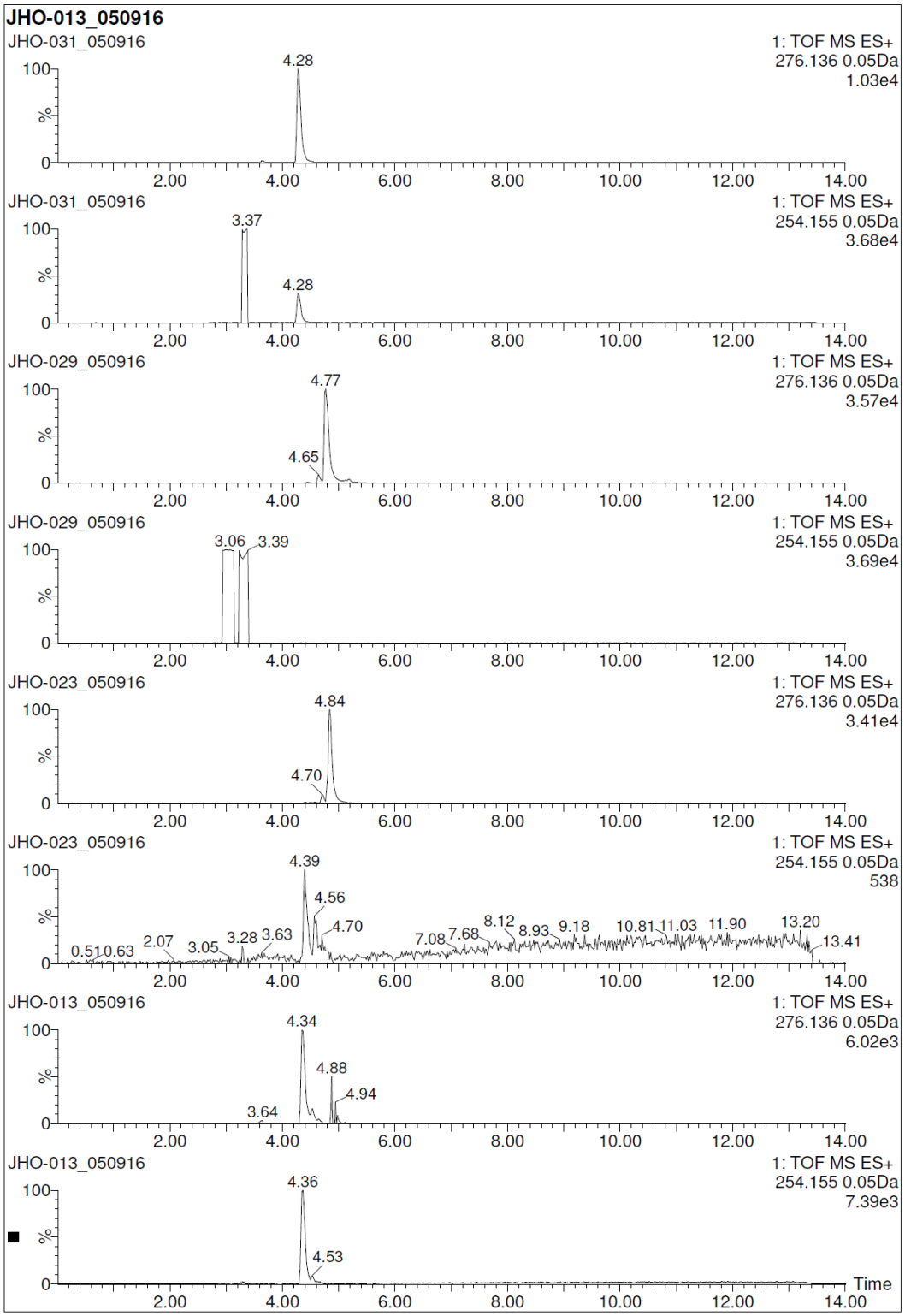
Acetylamphetamine 13 find mass MH MNa TIC



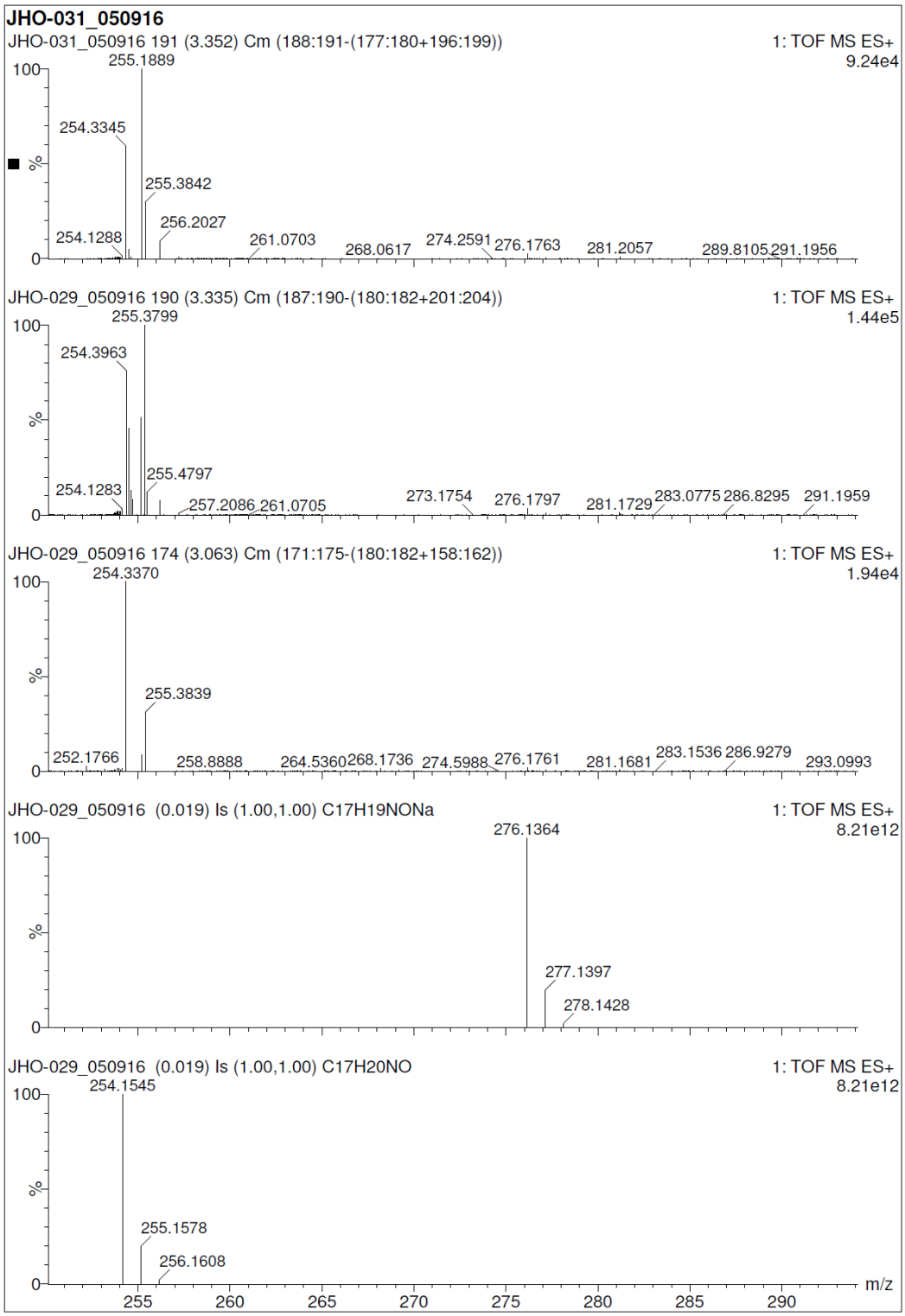
Acetylamphetamine 13 mass at retention time 3,64



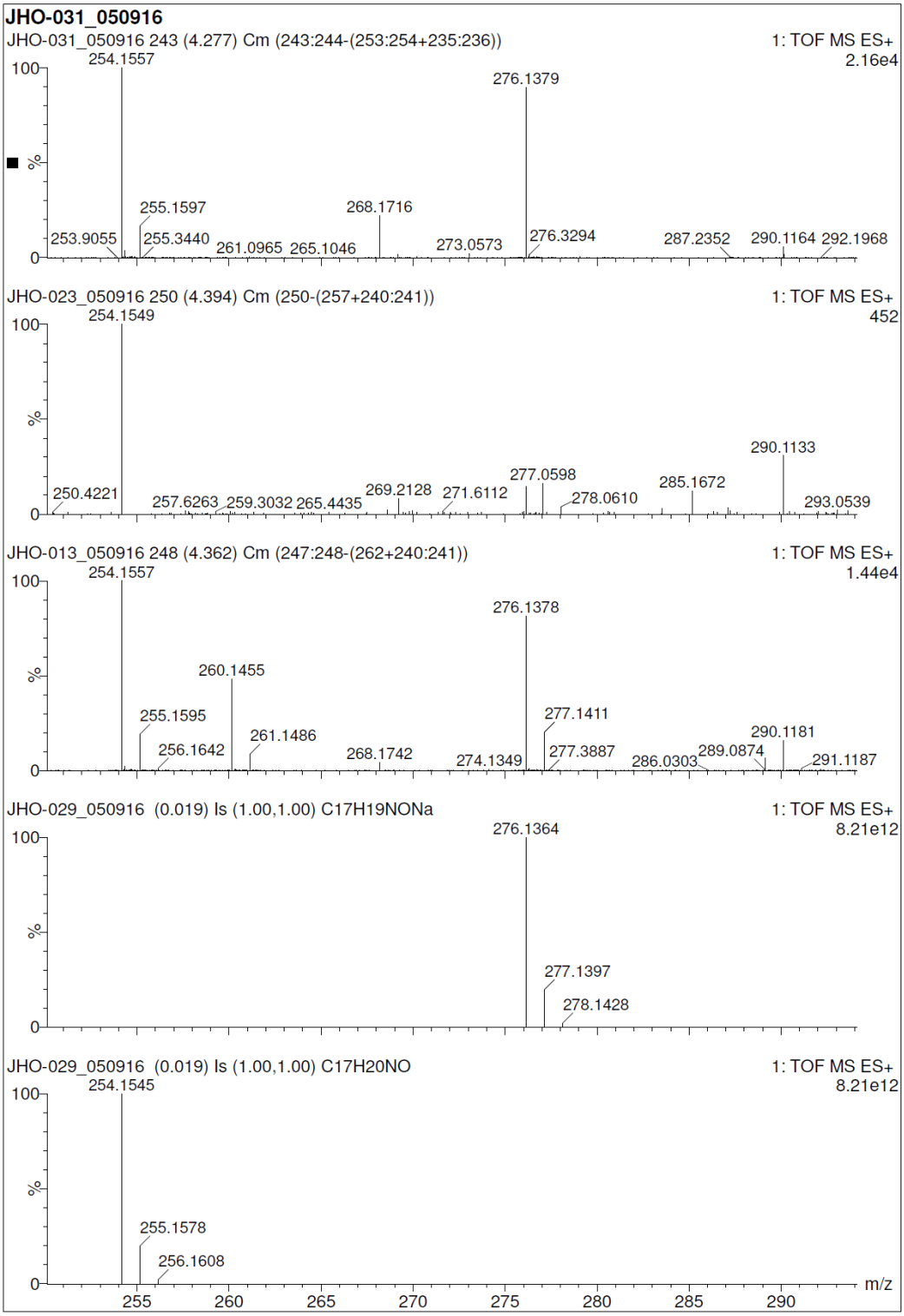
2-oxo-1-phenyl-(beta-phenylisopropylamino)etane 18 find mass MH MNa TIC



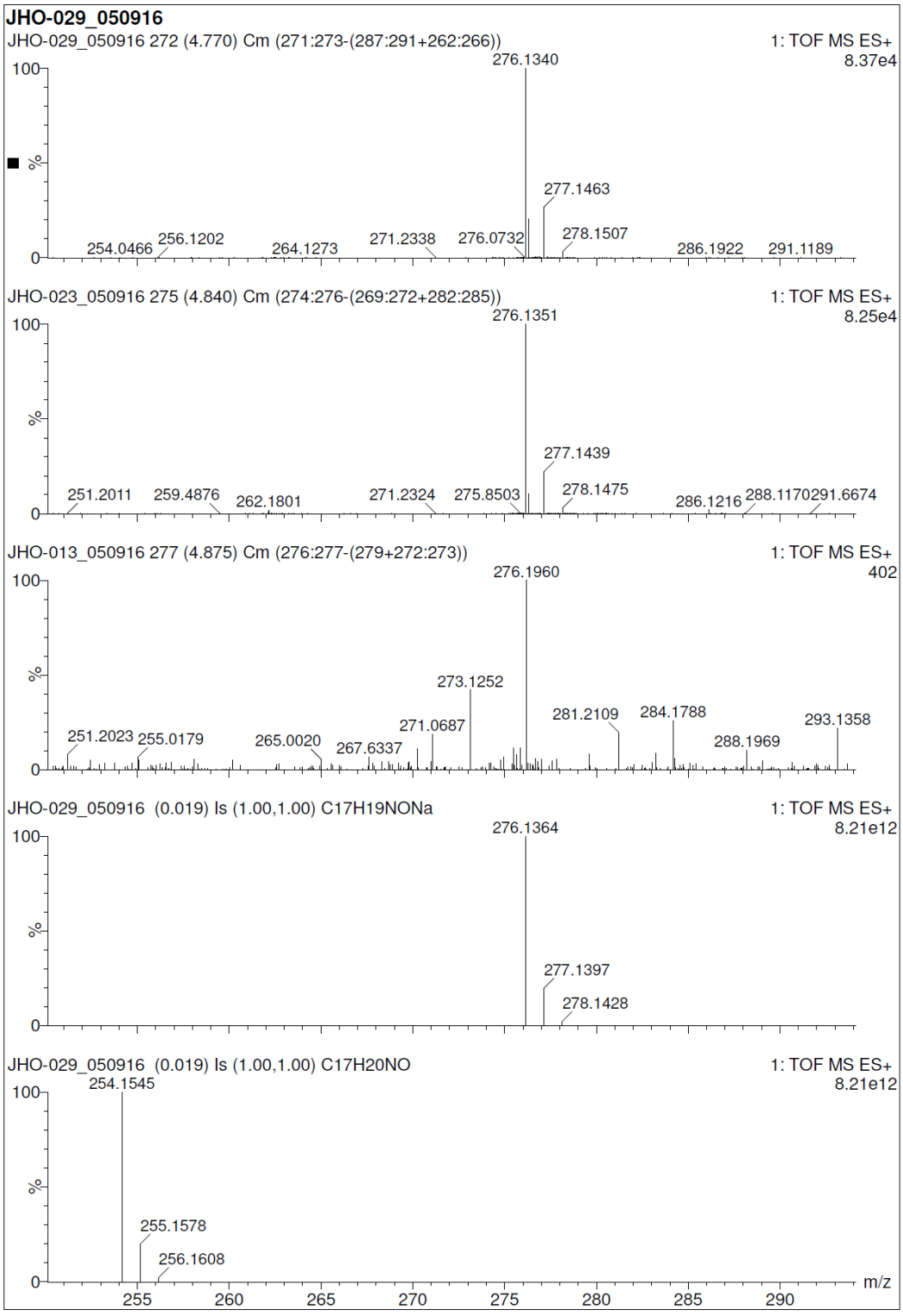
2-oxo-1-phenyl-(beta-phenylisopropylamino)etane 18 mass at retention time 3,06 and 3,37



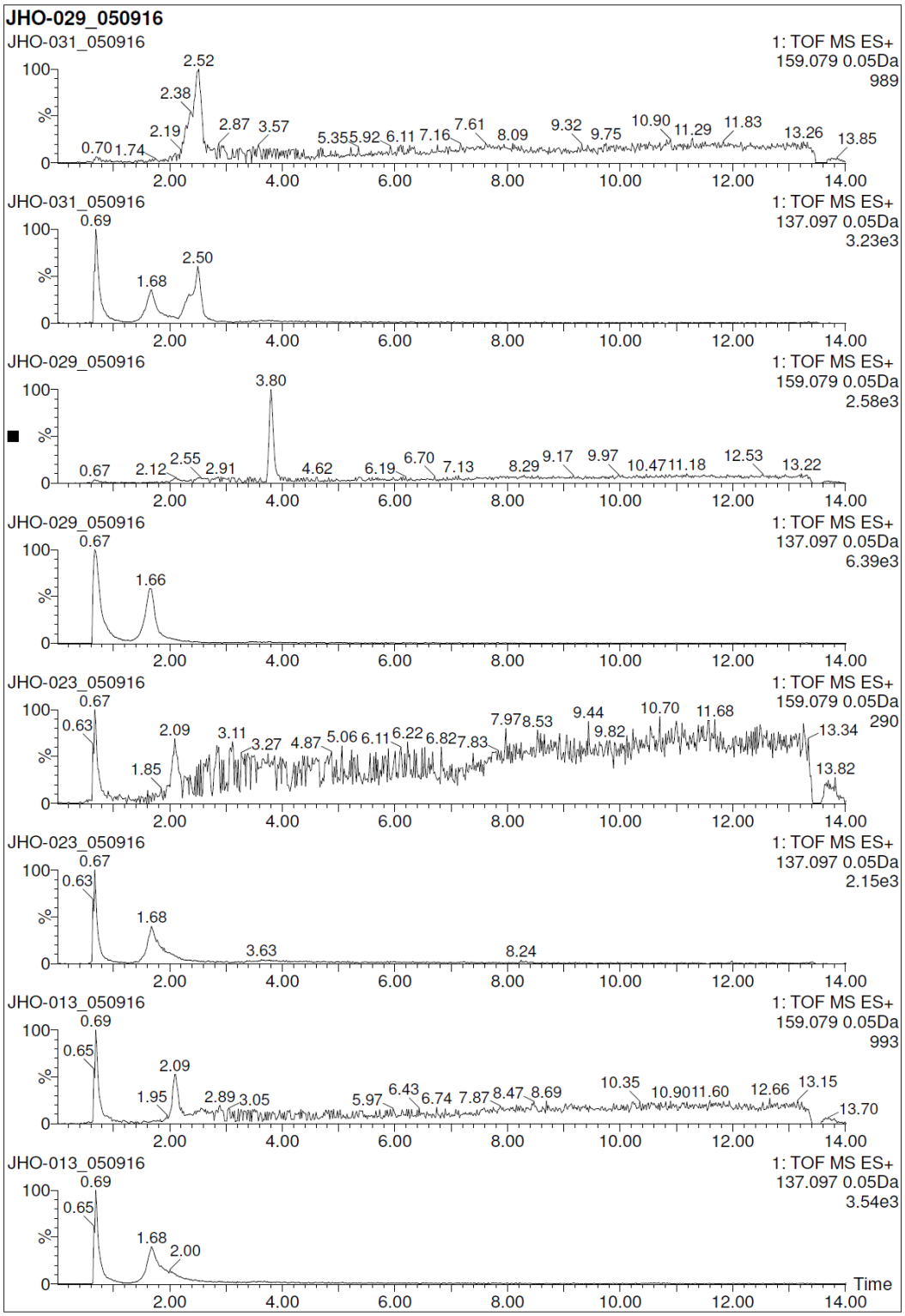
2-oxo-1-phenyl-(beta-phenylisopropylamino)etane 18 mass at retention time 4,28 and 4,34



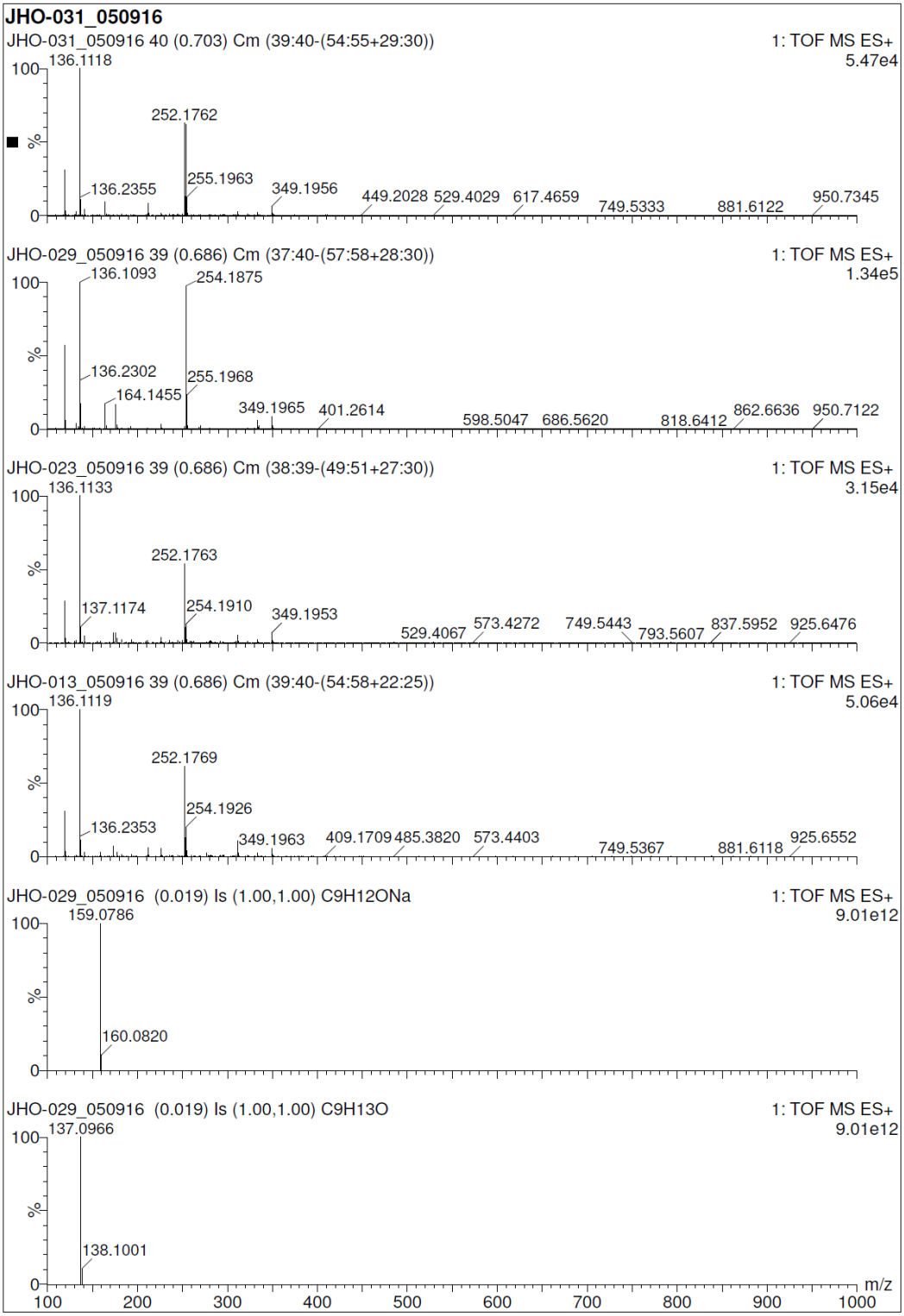
2-oxo-1-phenyl-(beta-phenylisopropylamino)etane 18 mass at retention time 4,77 and 4,84



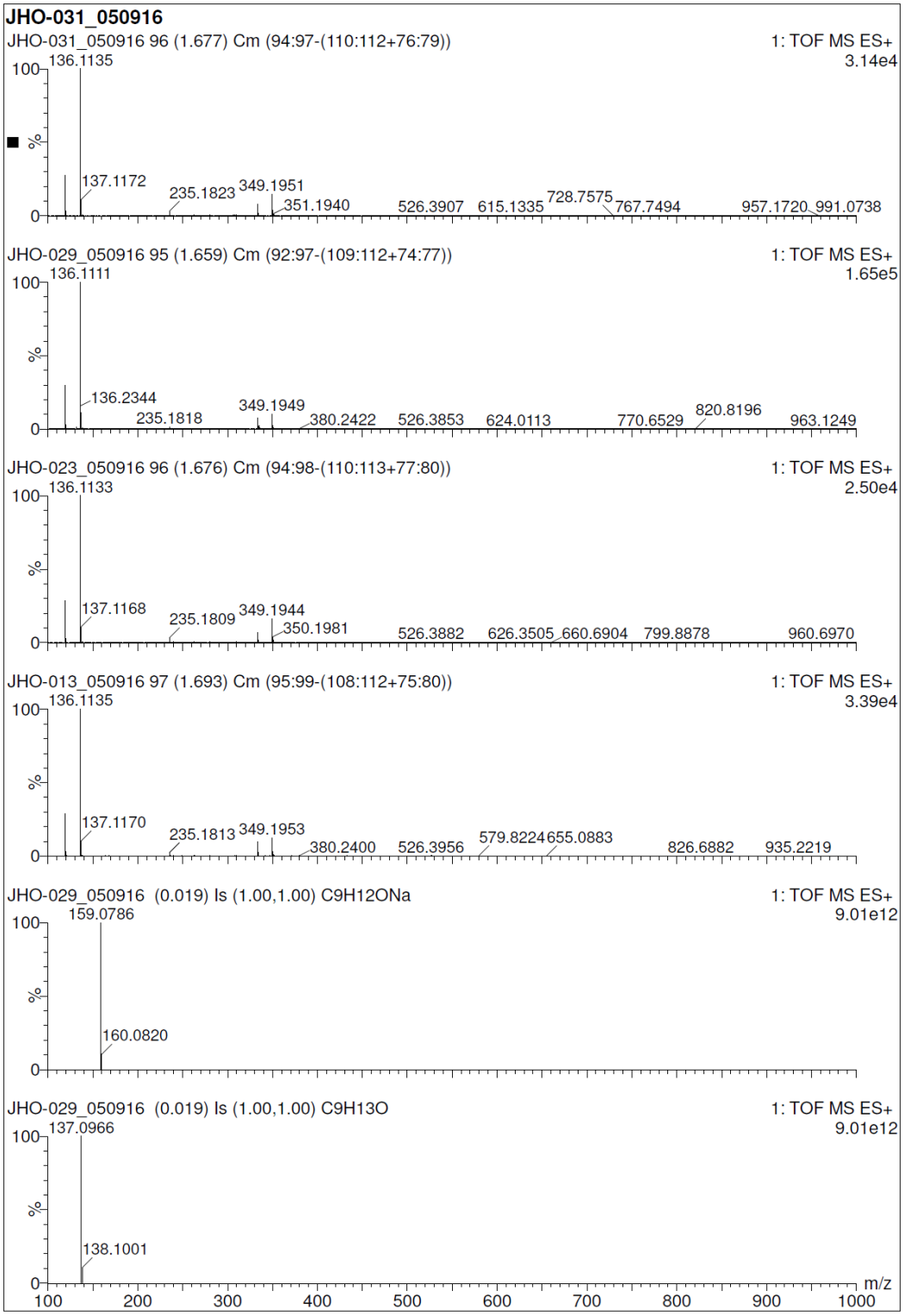
1-phenyl-2-propanol 16 find mass MH MNa TIC



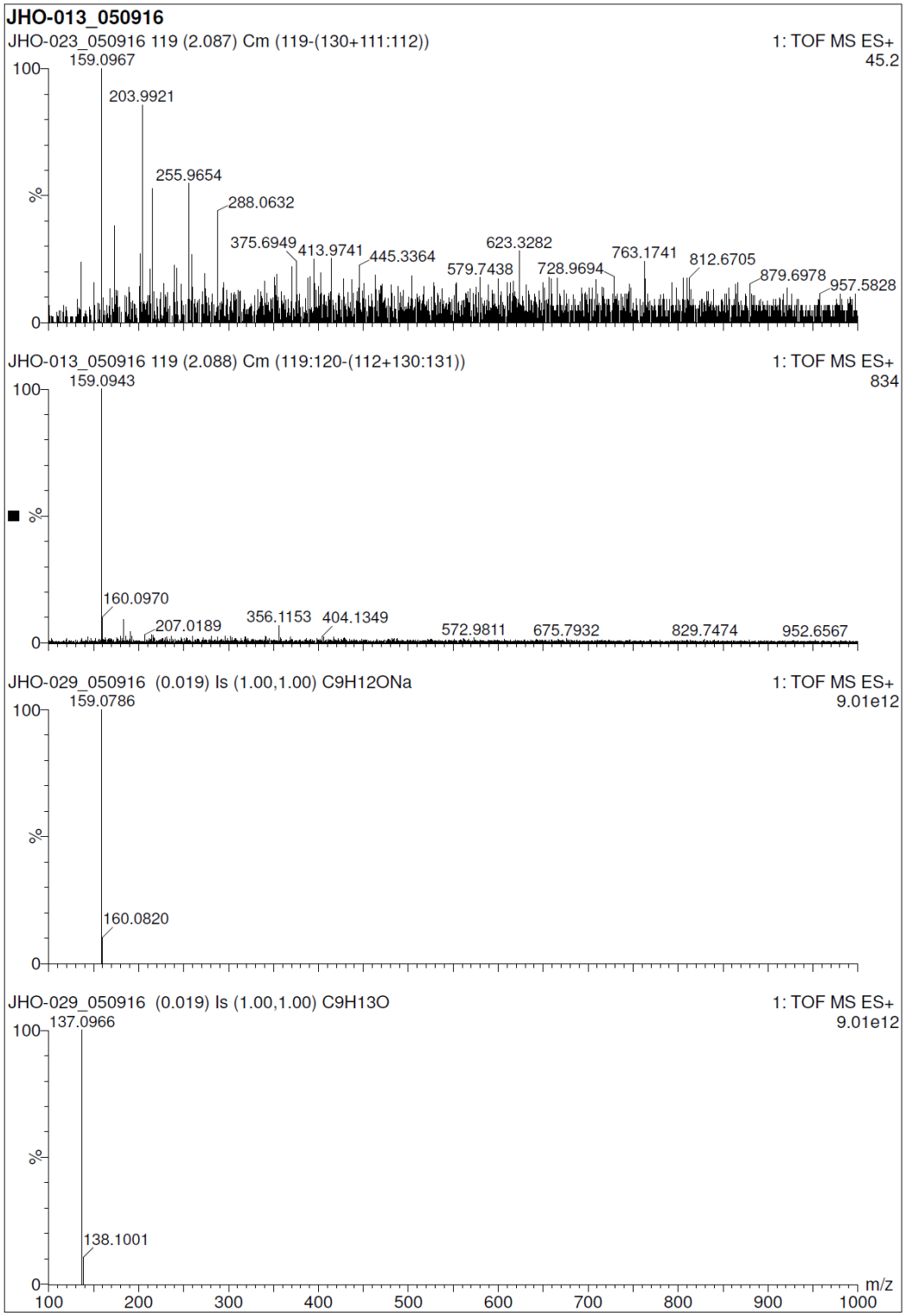
1-phenyl-2-propanol 16 mass at retention time 0,69



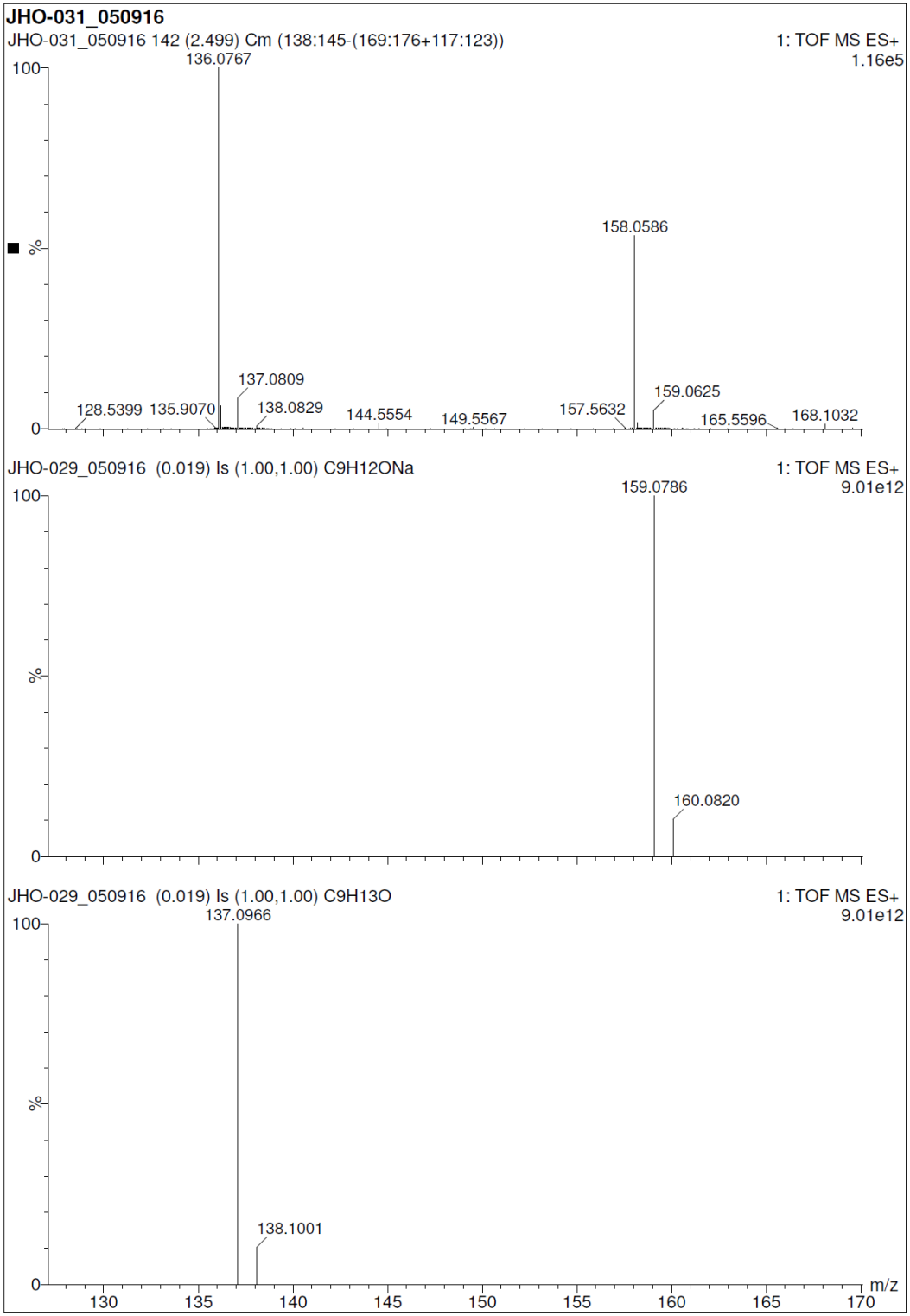
1-phenyl-2-propanol 16 mass at retention time 1,68



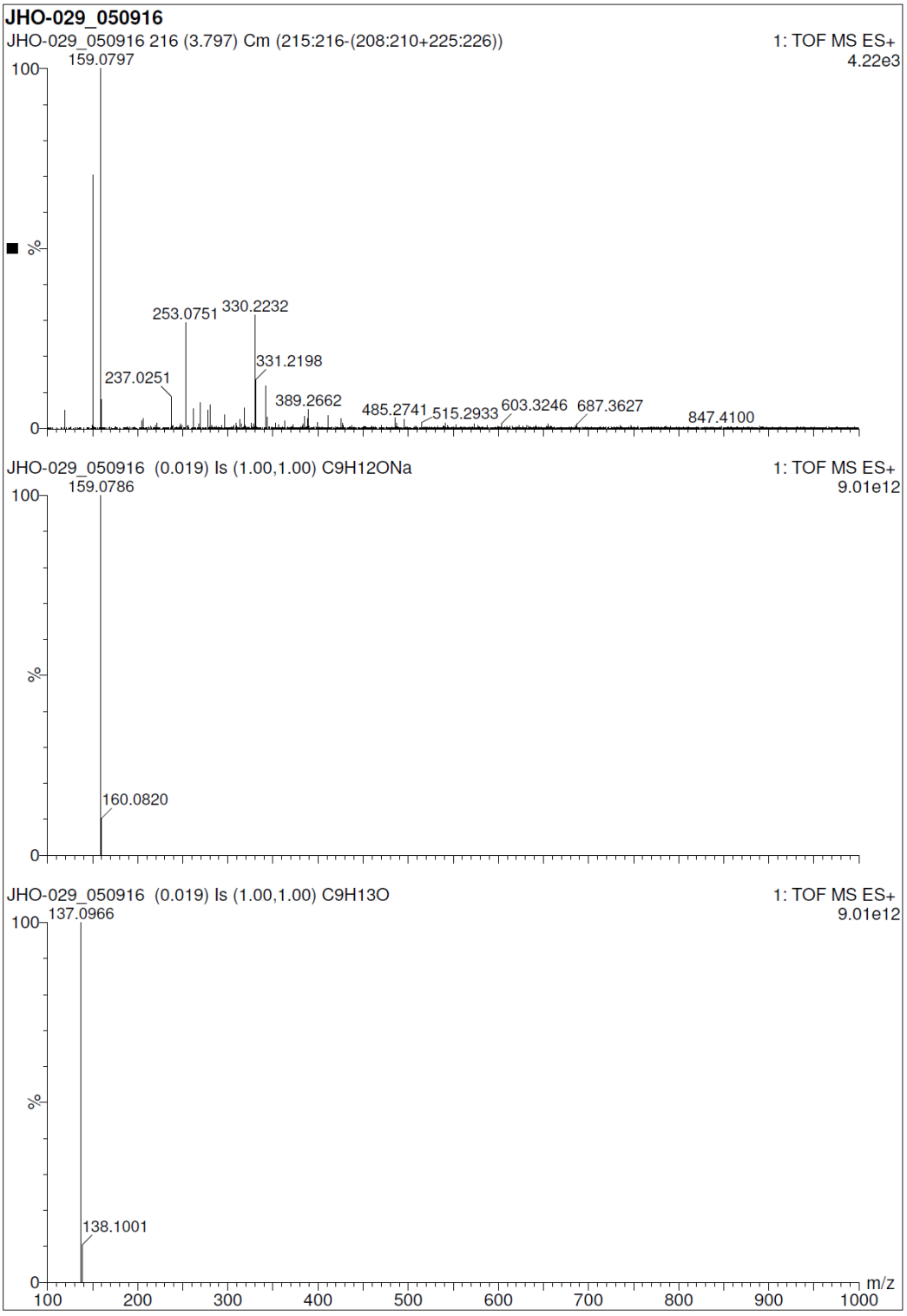
1-phenyl-2-propanol 16 mass at retention time 2,09



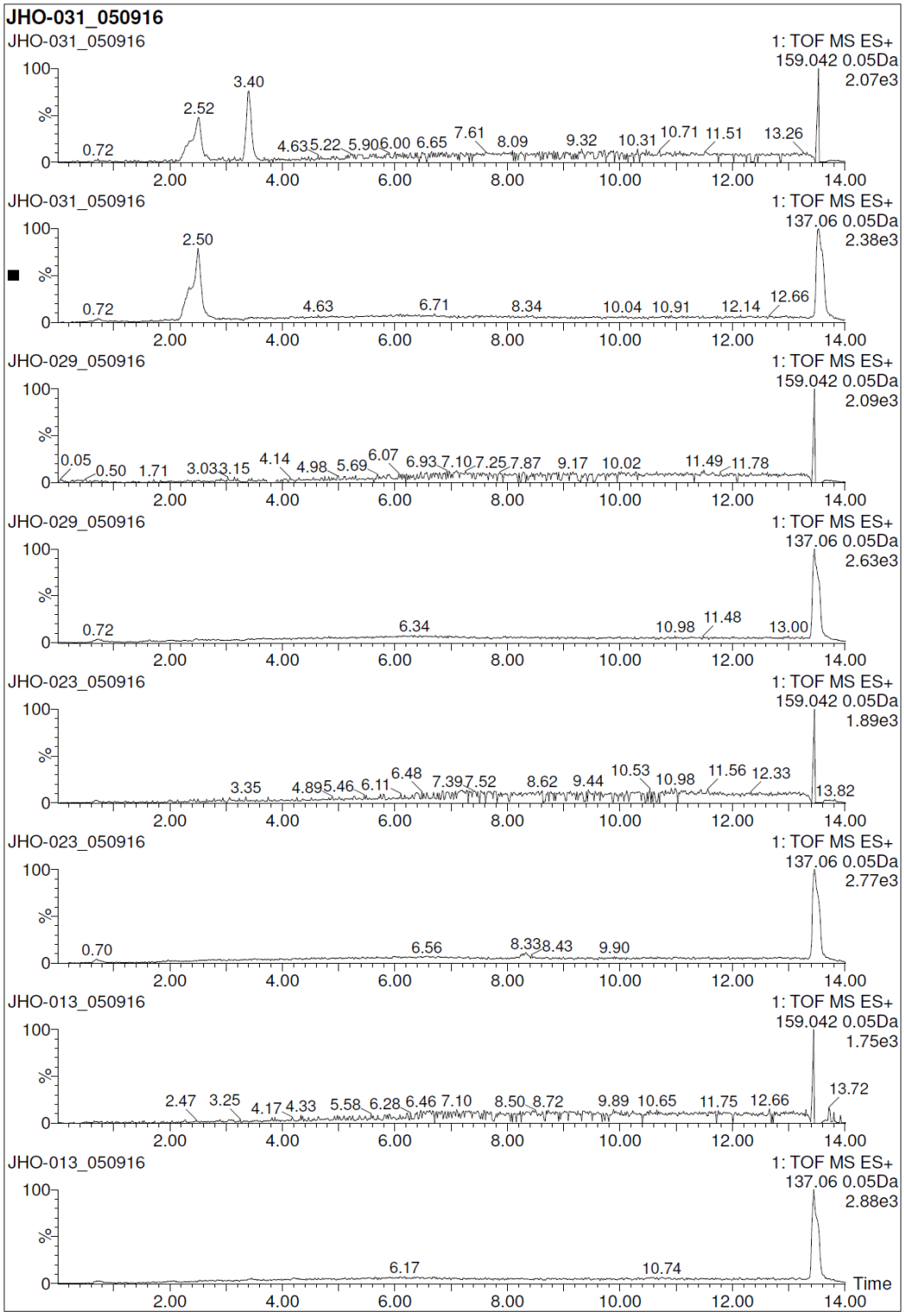
1-phenyl-2-propanol 16 mass at retention time 2,09



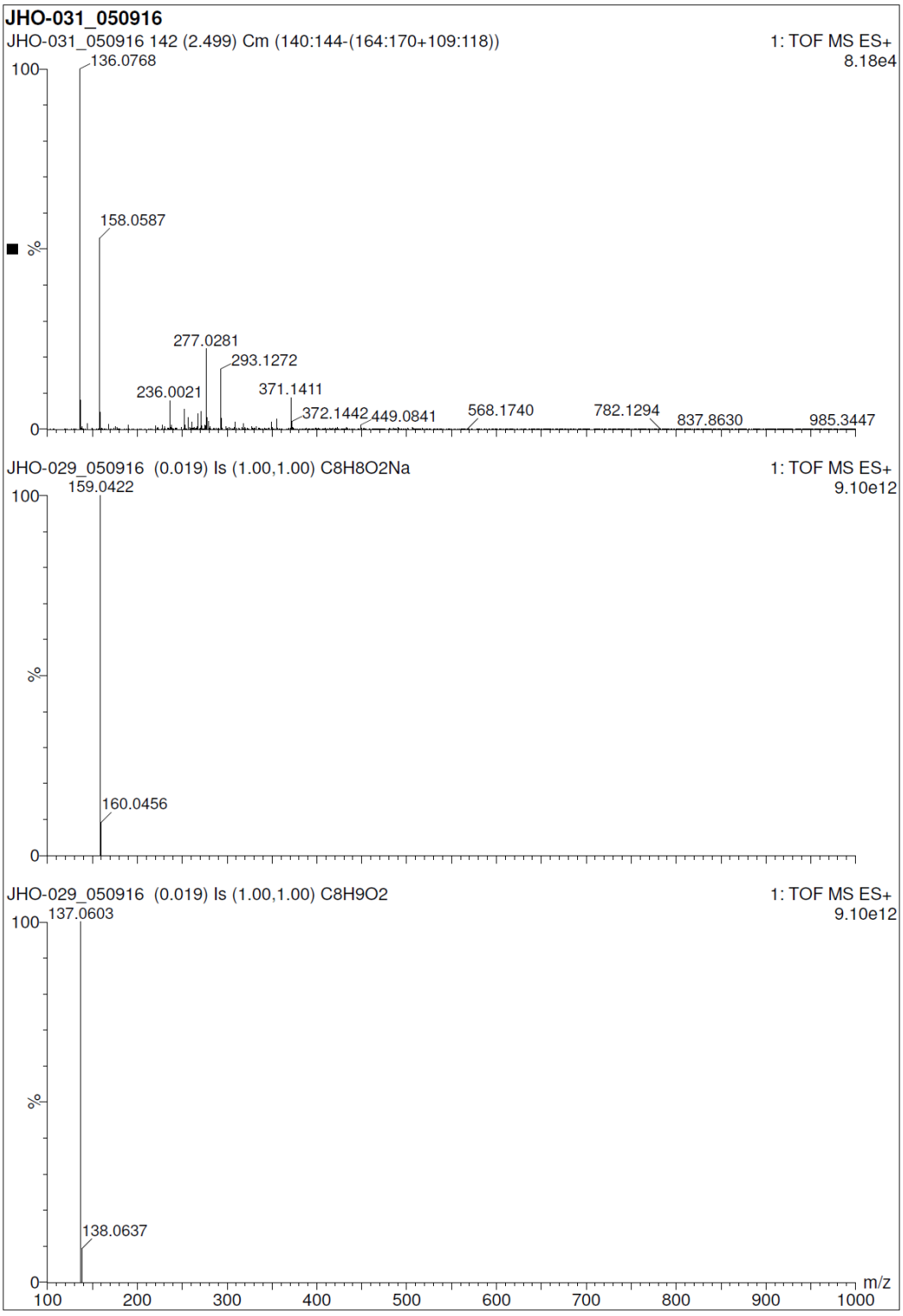
1-phenyl-2-propanol 16 mass at retention time 3,80



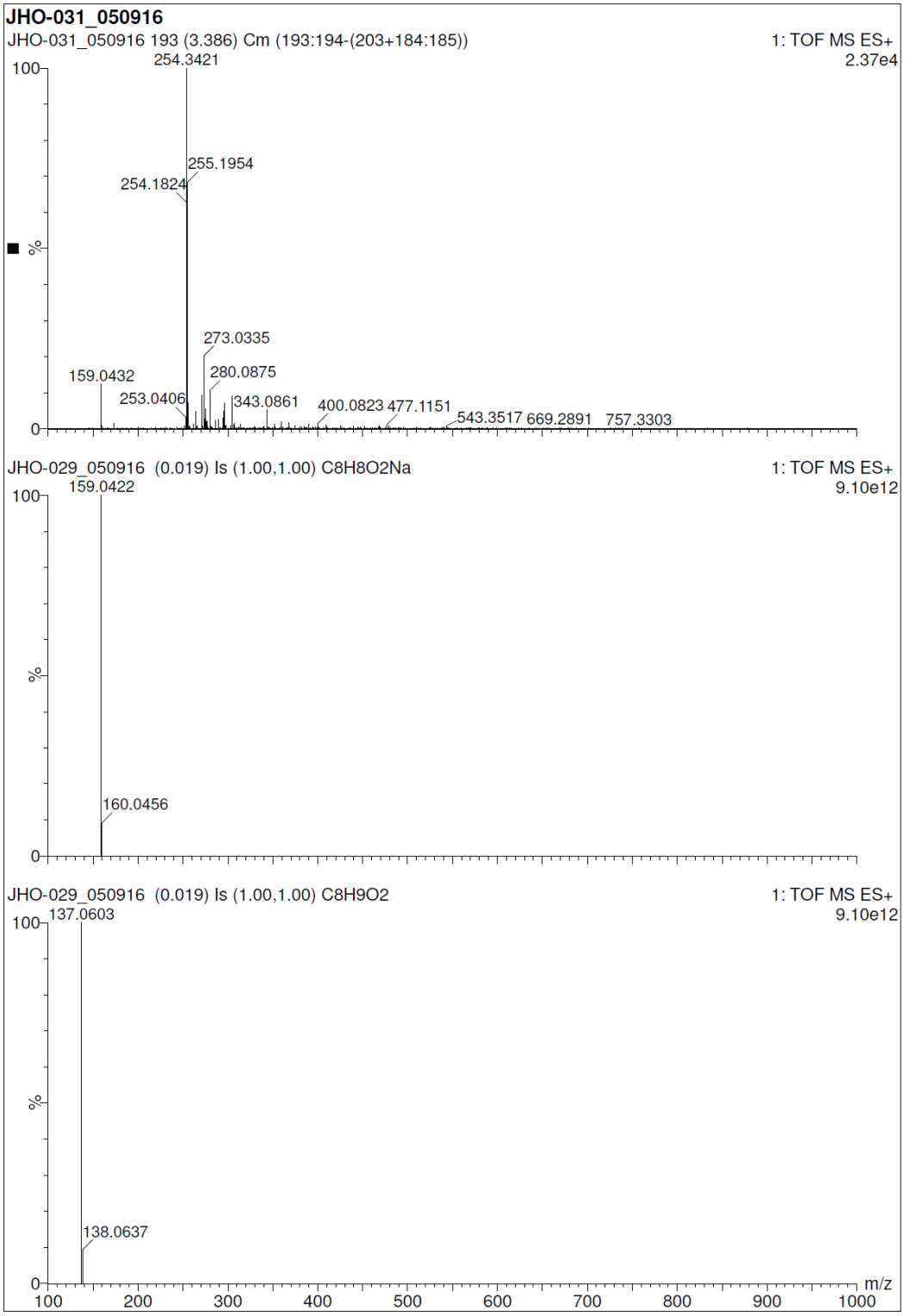
Acetic anhydride 8 find mass MH MNa TIC



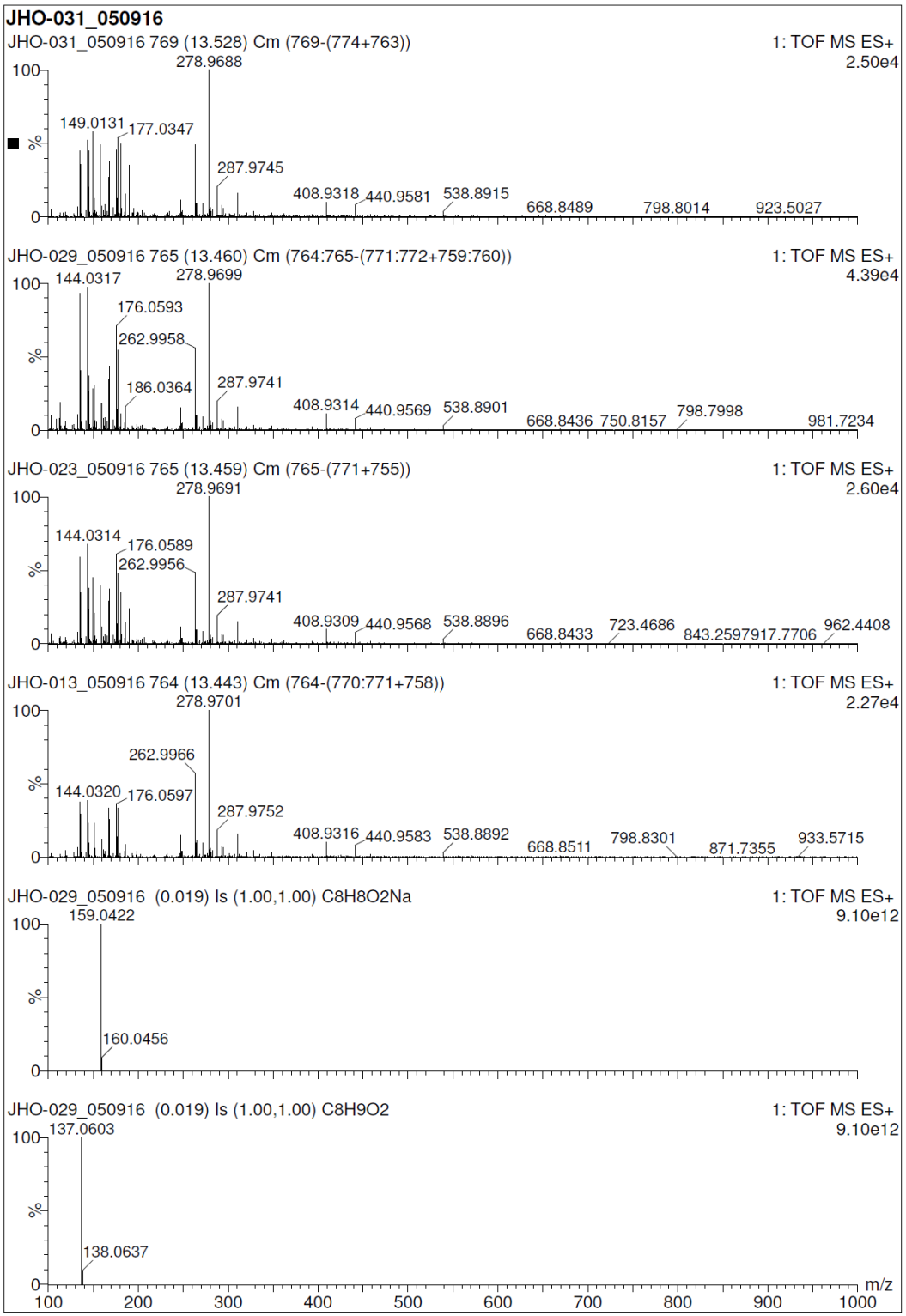
Acetic anhydride 8 mass at retention time 2,50



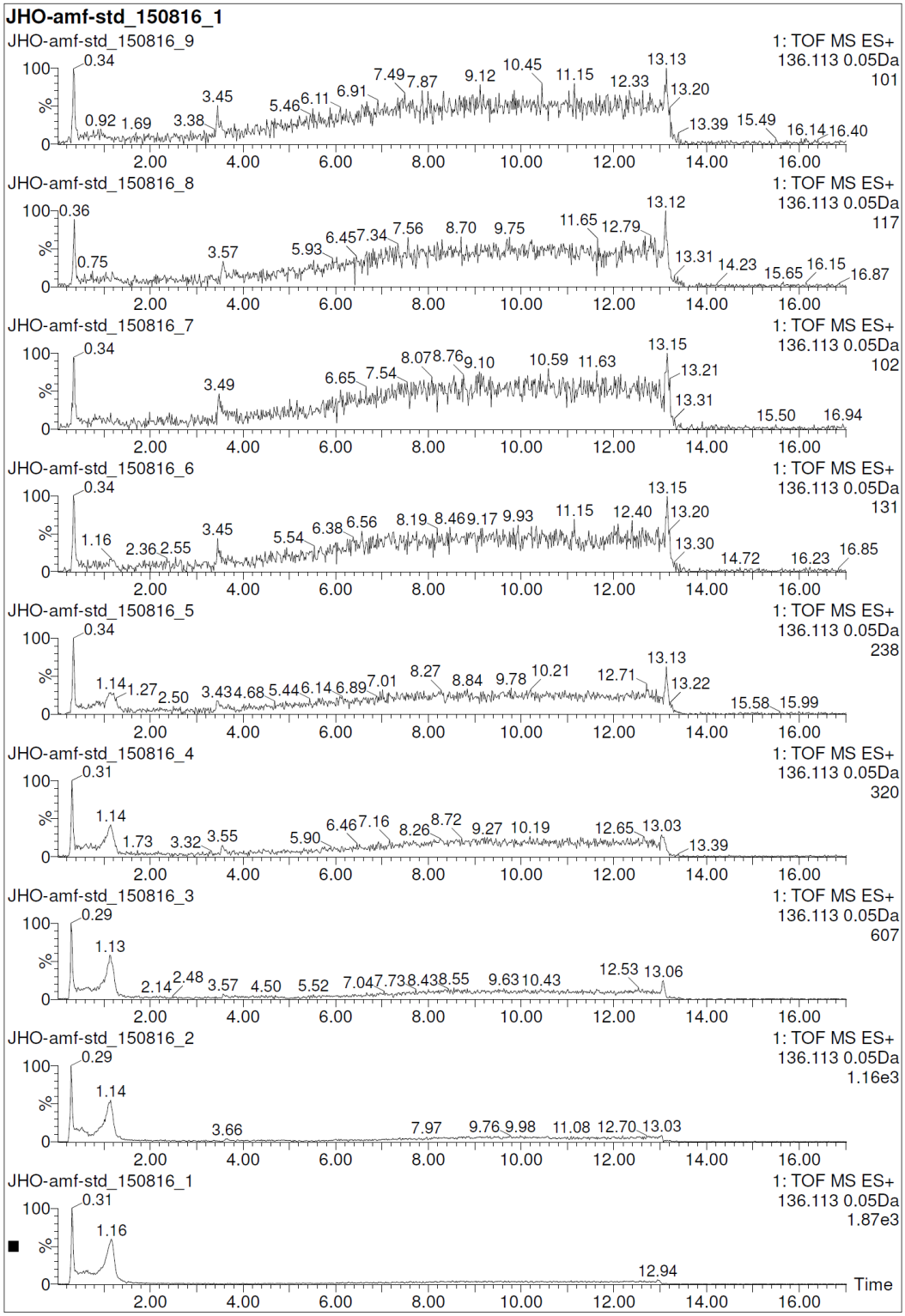
Acetic anhydride 8 mass at retention time 3,40



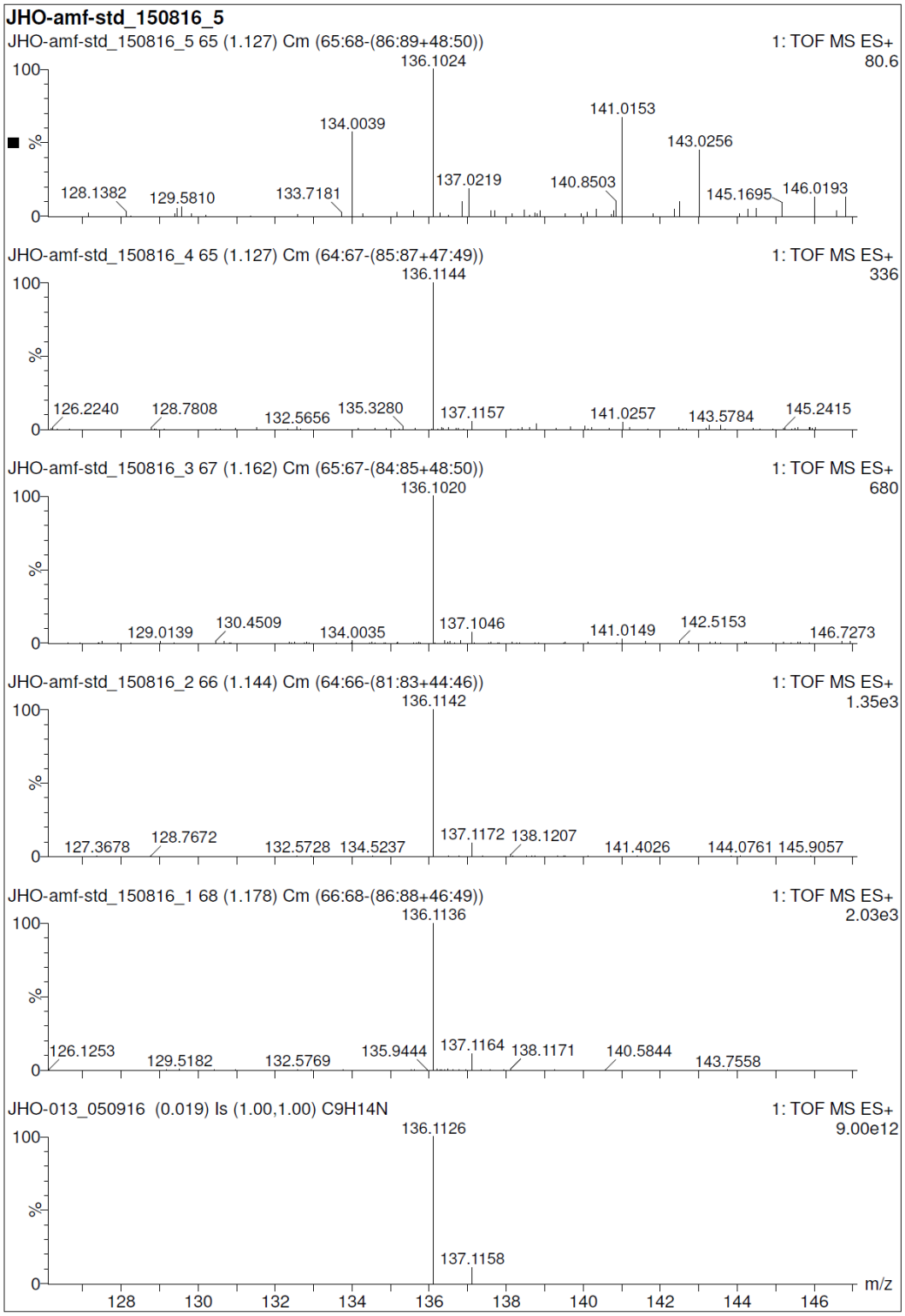
Acetic anhydride 8 mass at retention time 3,40



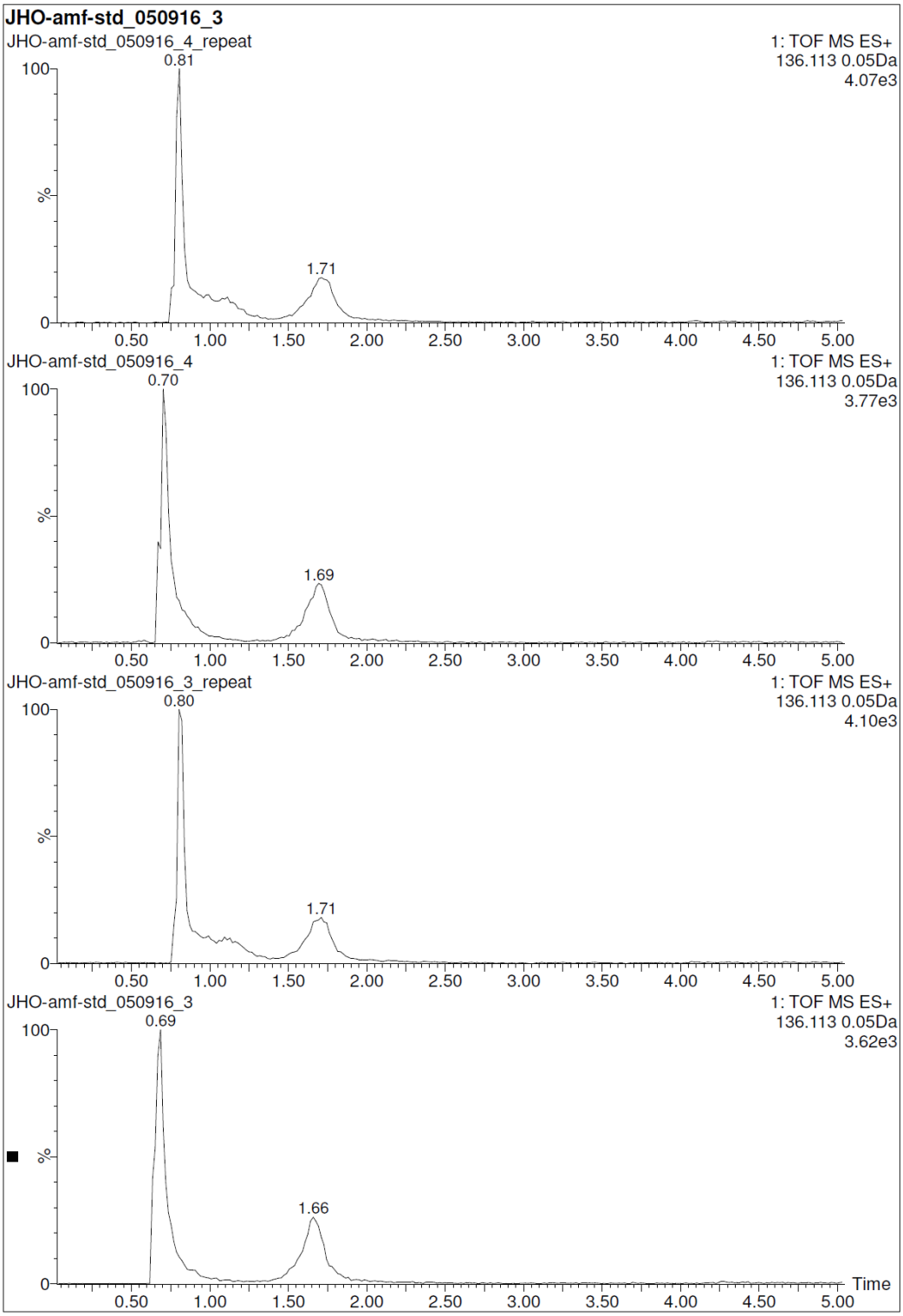
Amphetamine standard run 150816 find mass MH TIC



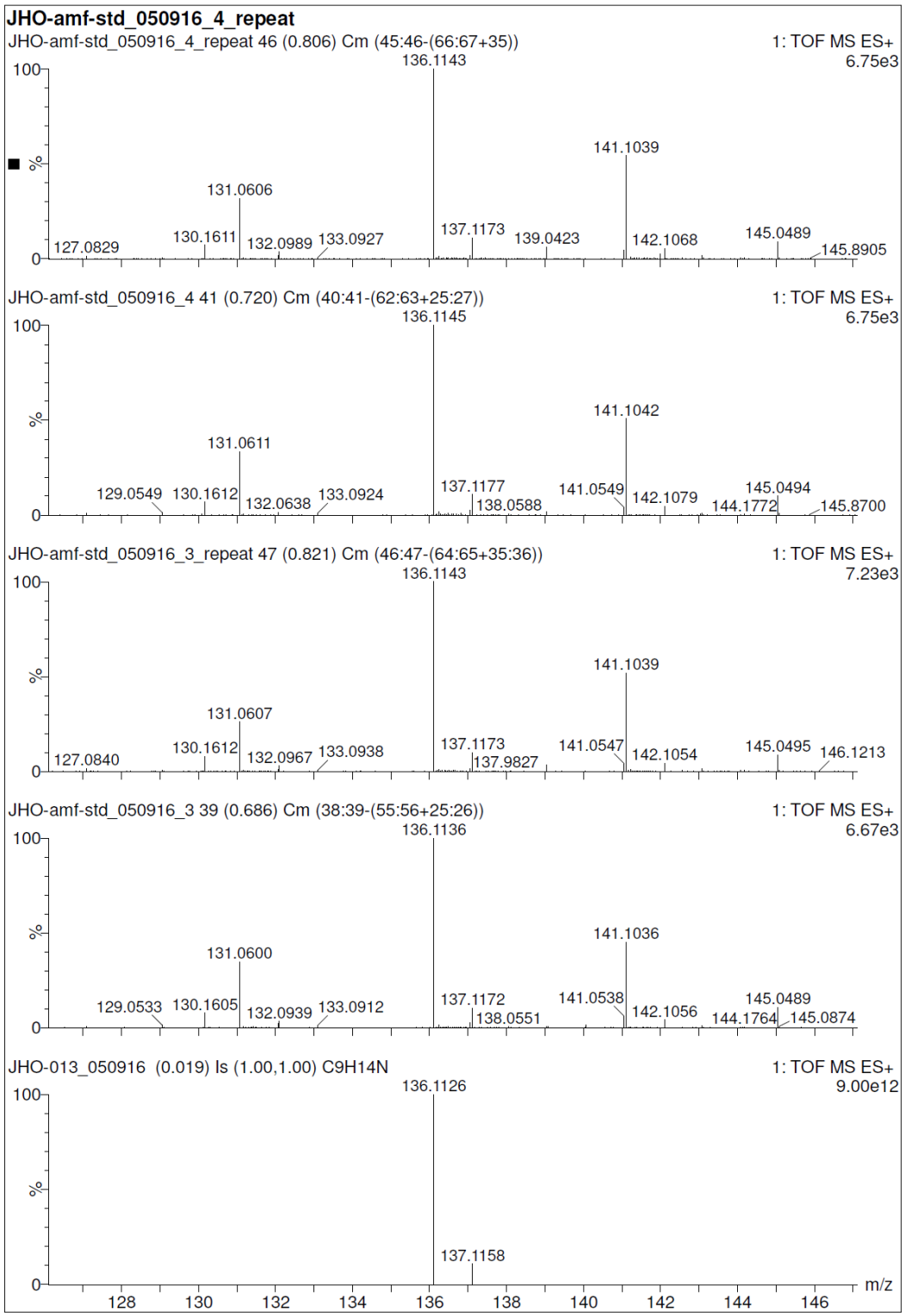
Amphetamine standard run 150816 mass for retention time 1,13



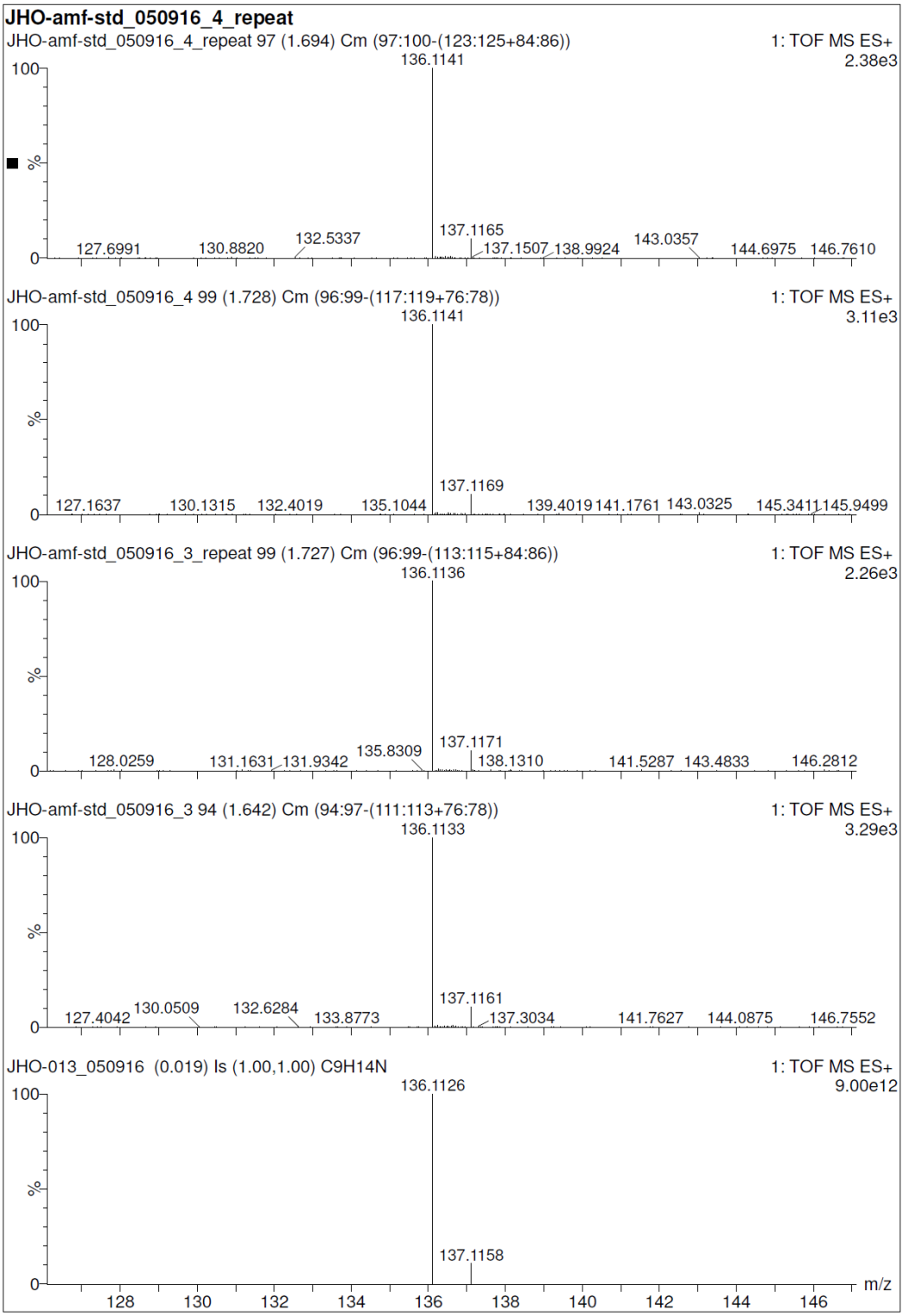
Amphetamine standard on the experiment day find mass MH TIC



Amphetamine standard on the experiment mass at retention time 0,69

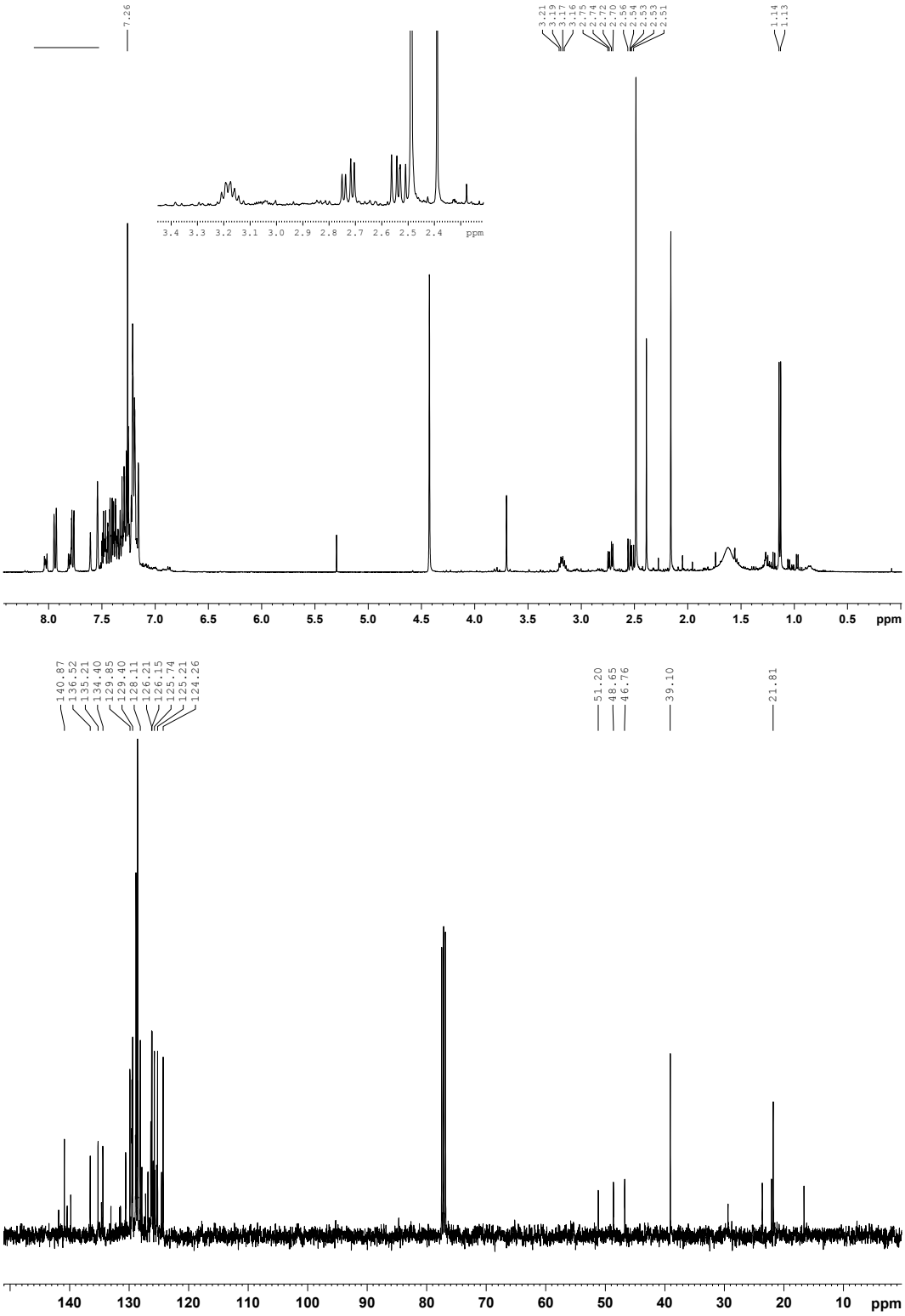


Amphetamine standard on the experiment mass at retention time 1,70

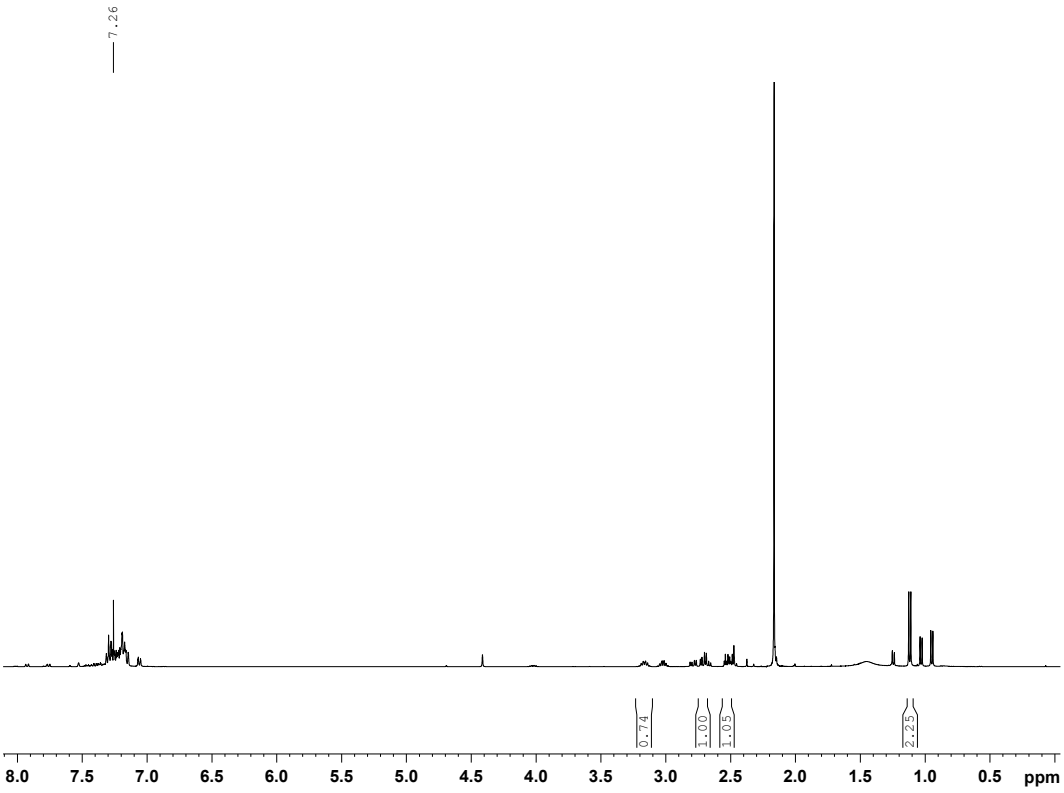


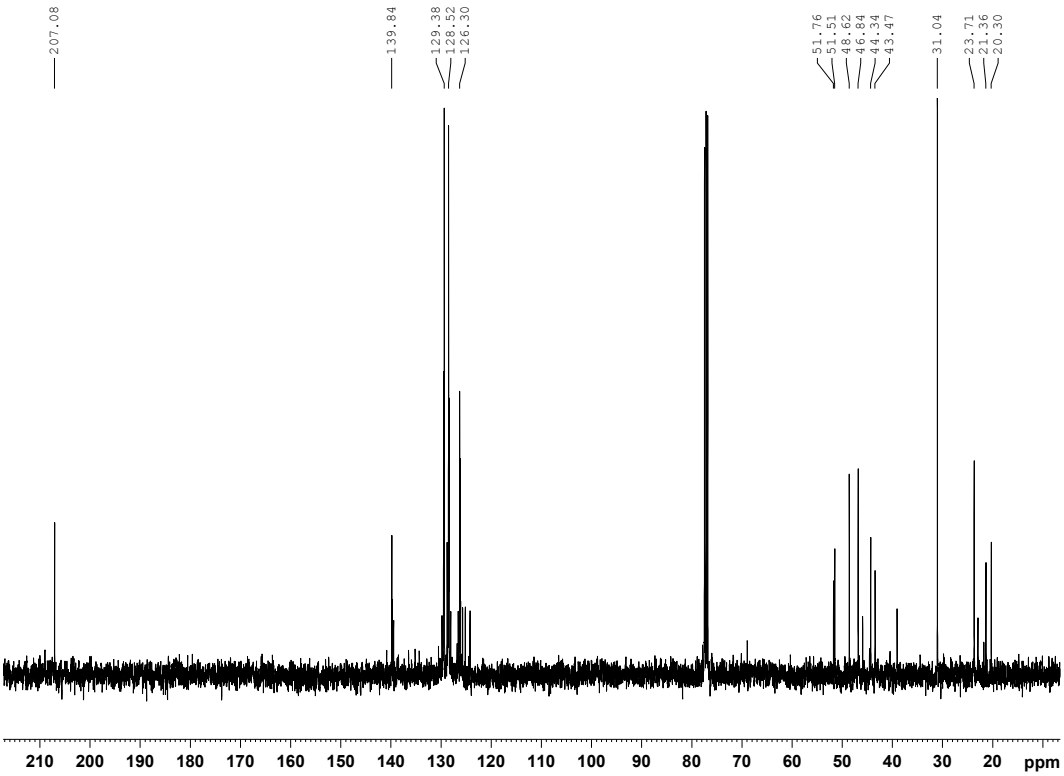
Appendix 7: ^{1}H and ^{13}C NMR spectra for the synthesized substances

Amphetamine I crude from route I ^{1}H and ^{13}C NMR spectra

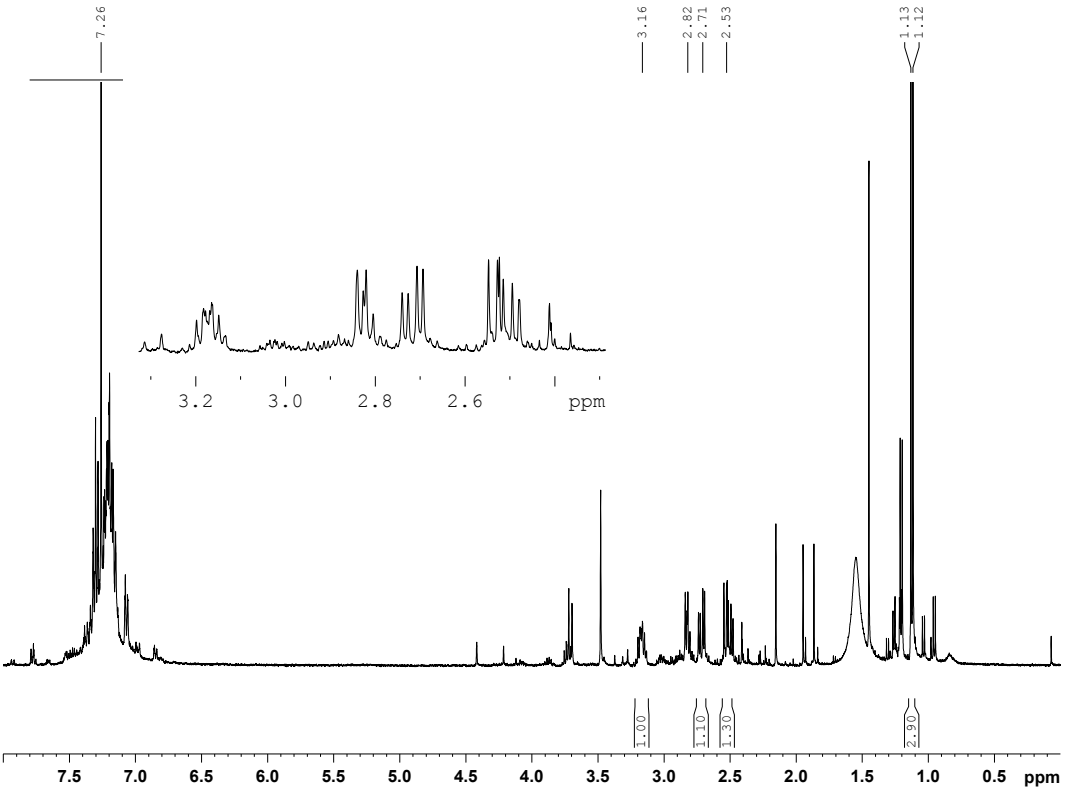


Amphetamine I crude from route II ^{1}H and ^{13}C NMR spectra

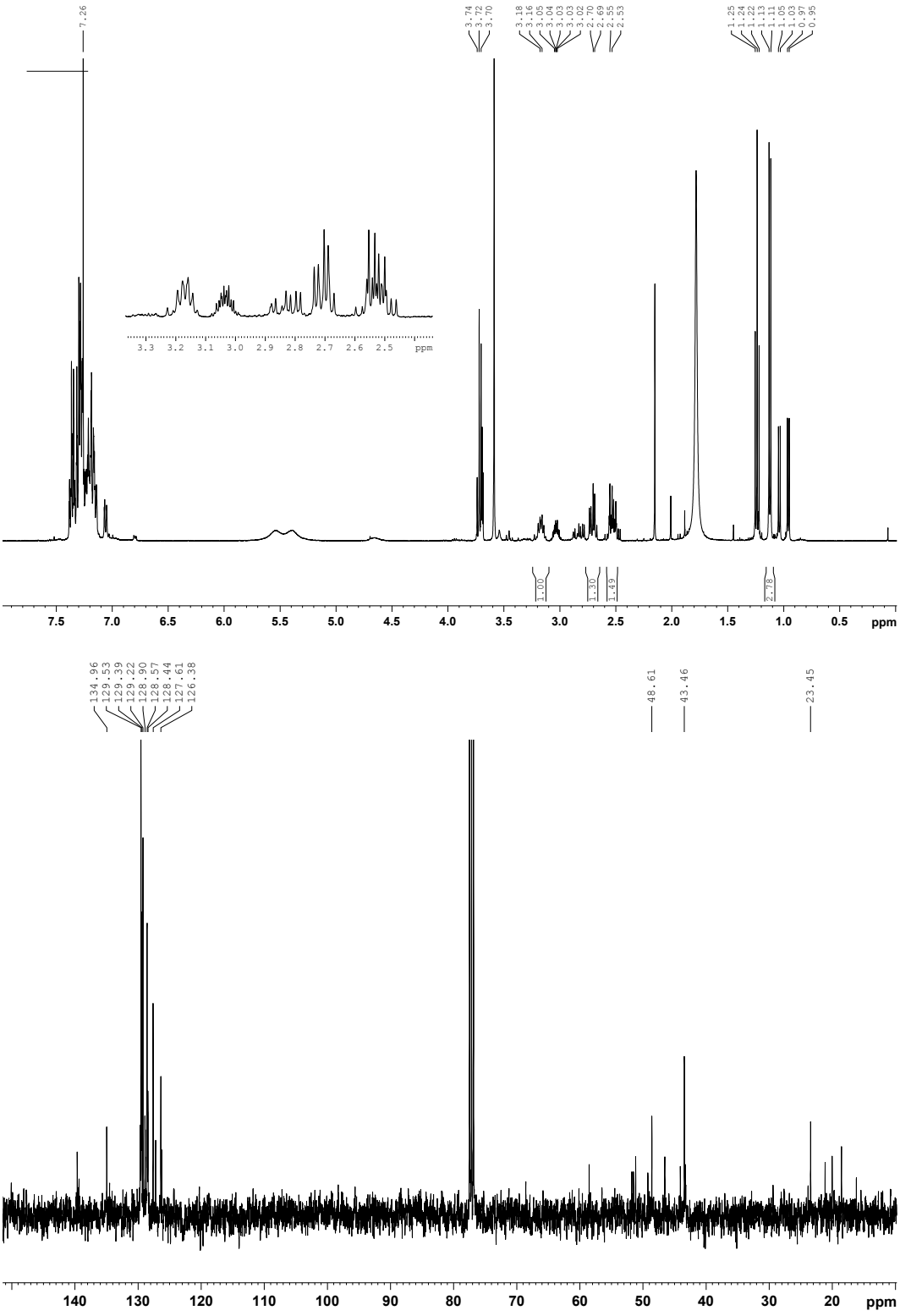




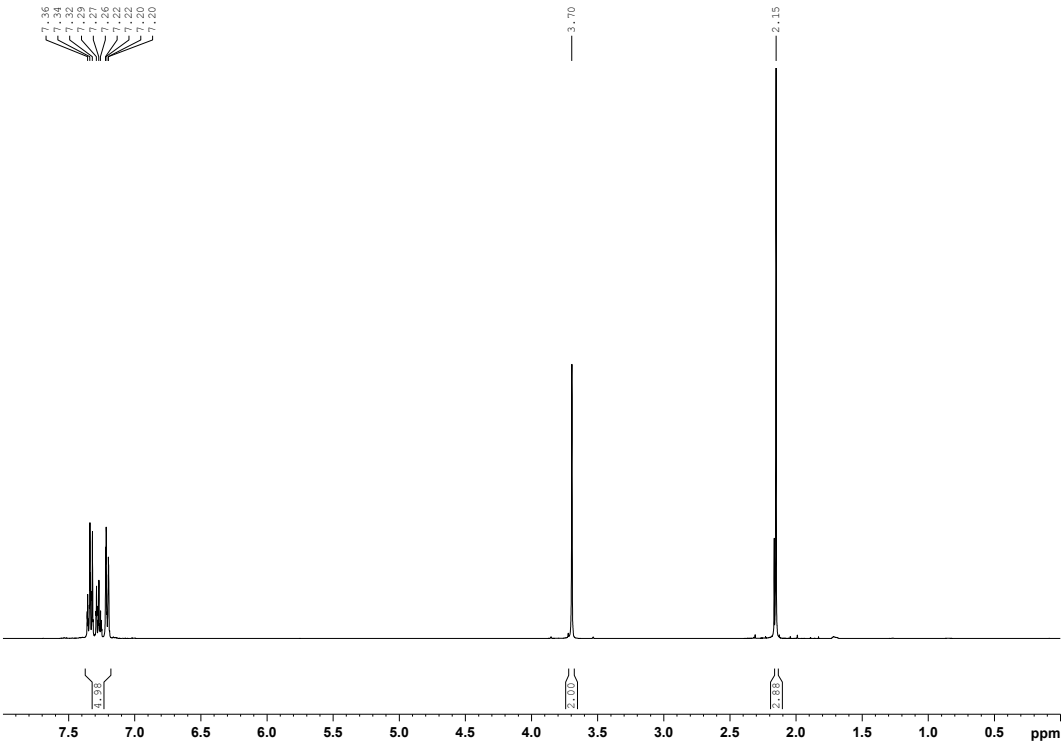
Amphetamine I crude from route III ^{1}H NMR spectra

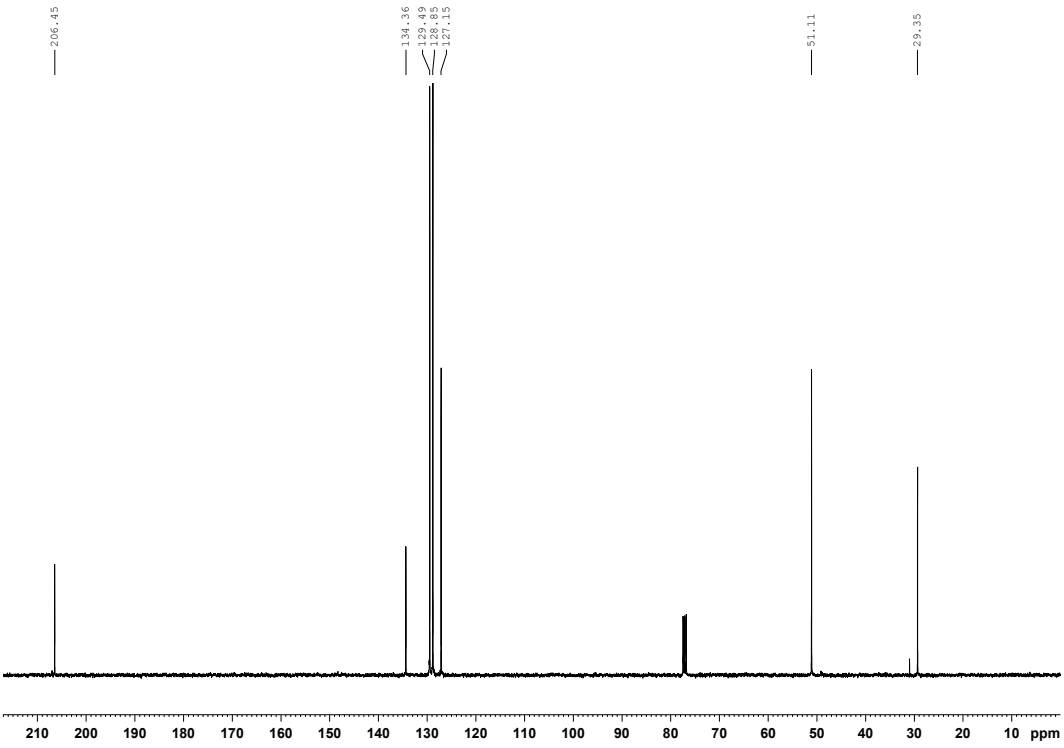


Amphetamine I crude from route IV ^{1}H NMR spectra

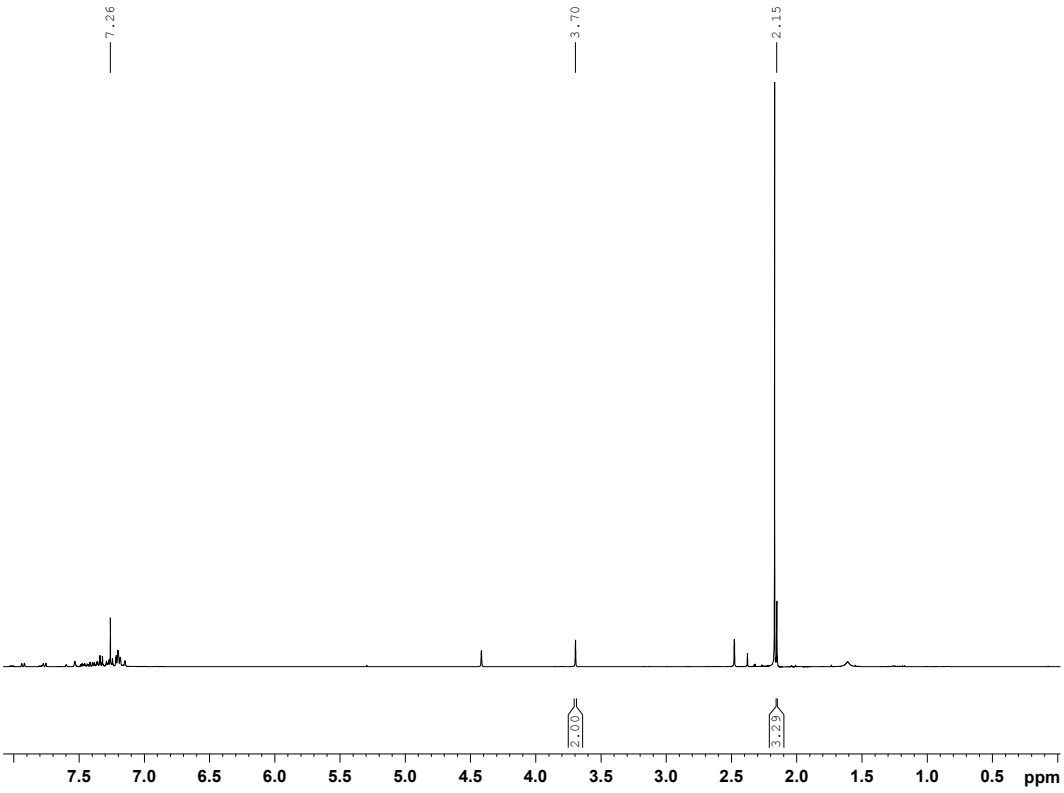


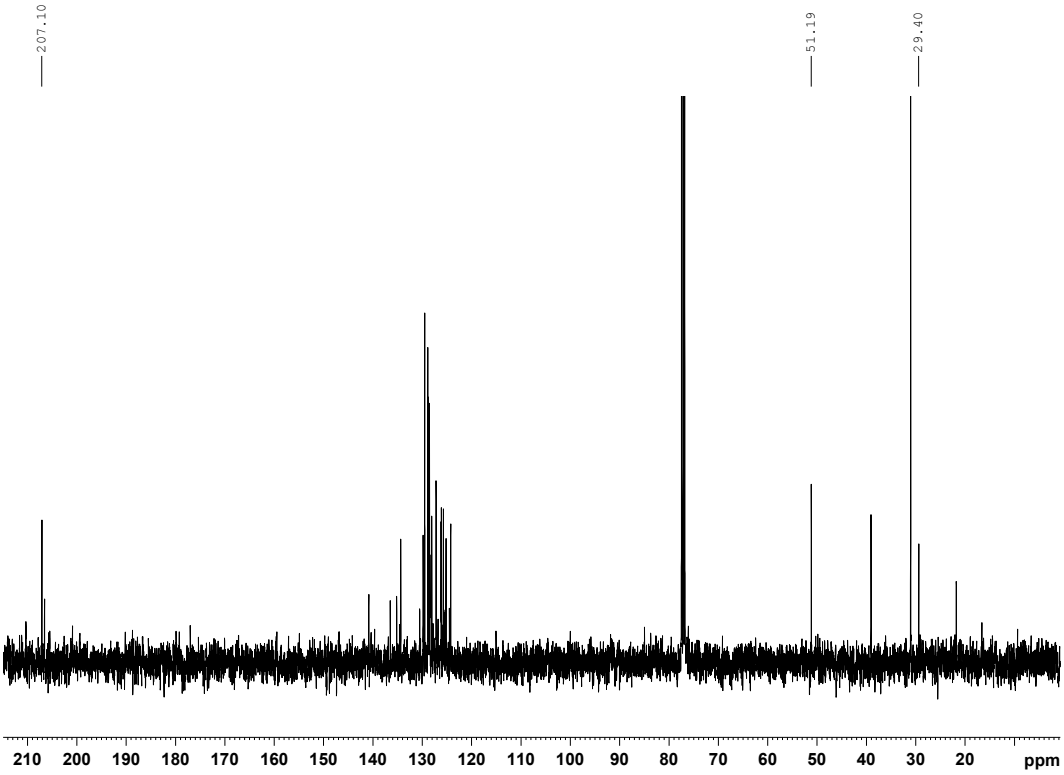
Purified P2P 5 ^{1}H and ^{13}C NMR spectra



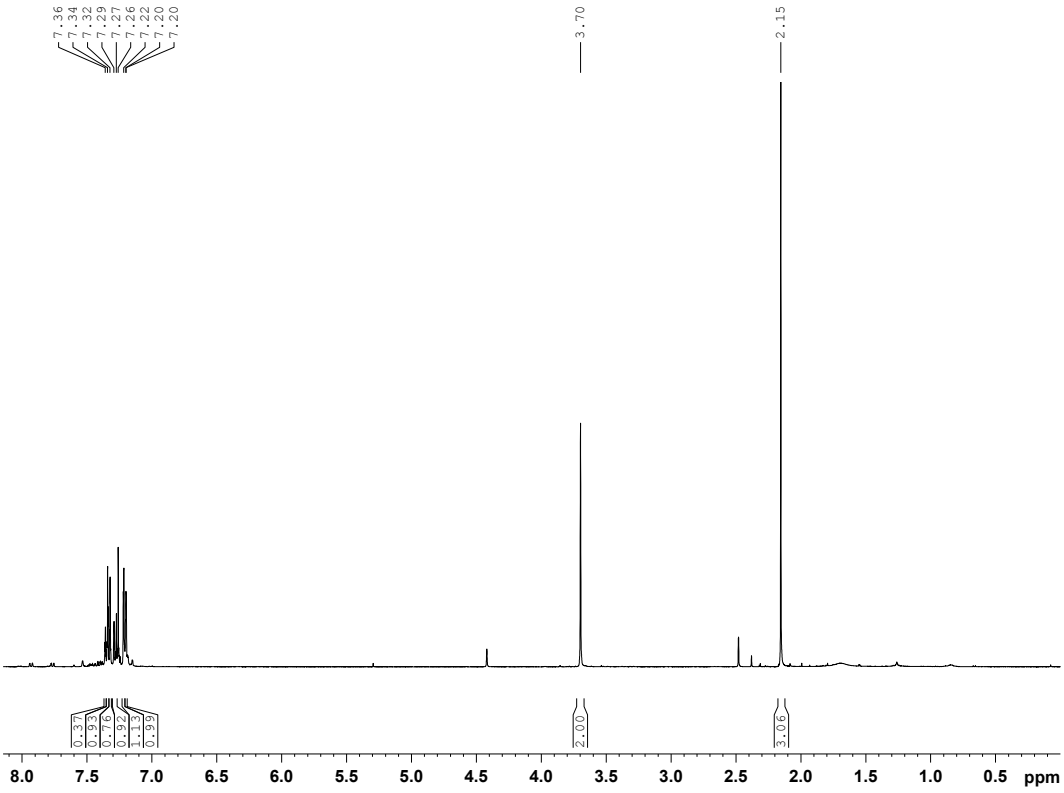


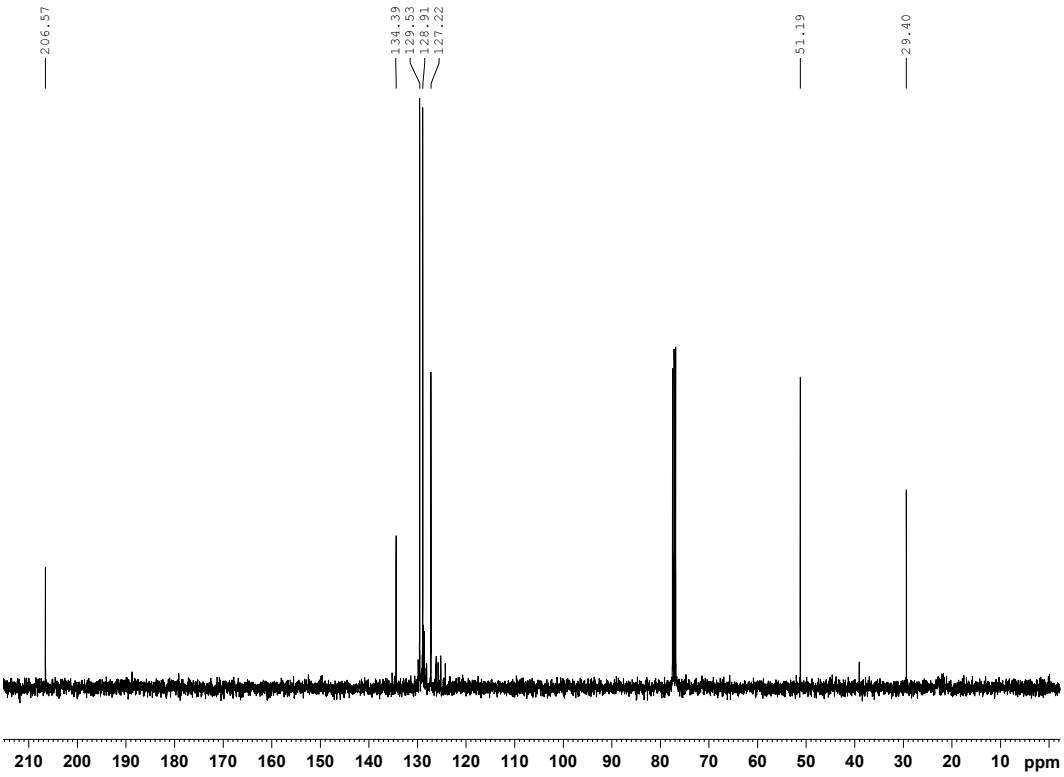
P2P 5 crude from route I ^{1}H and ^{13}C NMR spectra



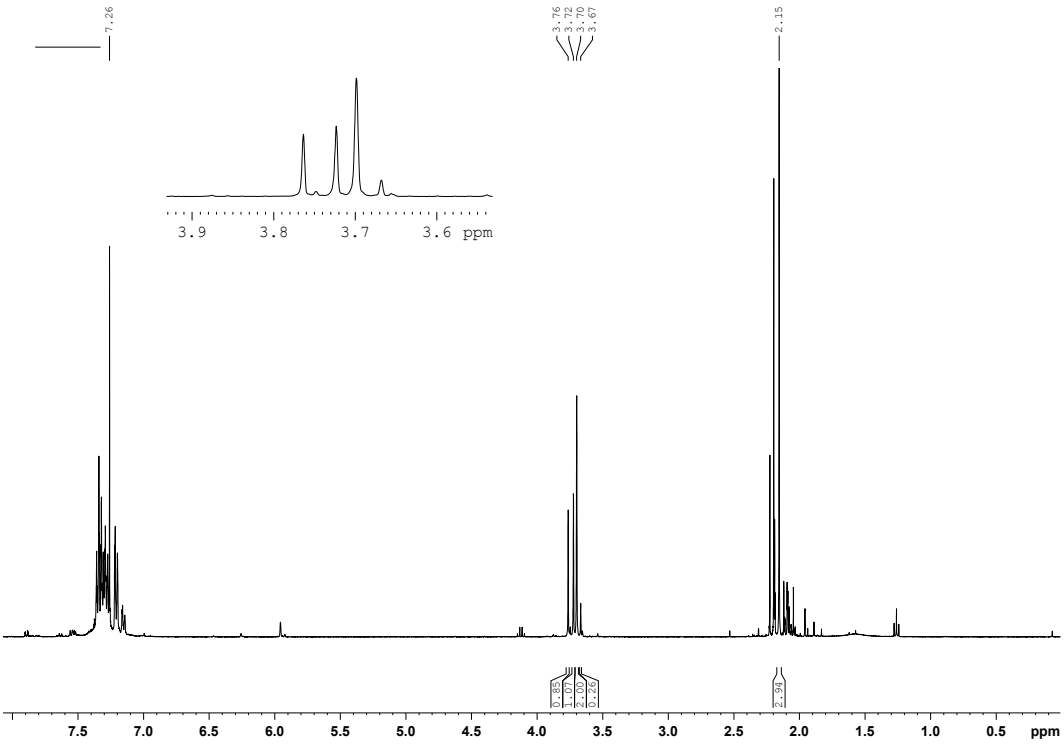


P2P 5 crude from route II ^{1}H and ^{13}C NMR spectra

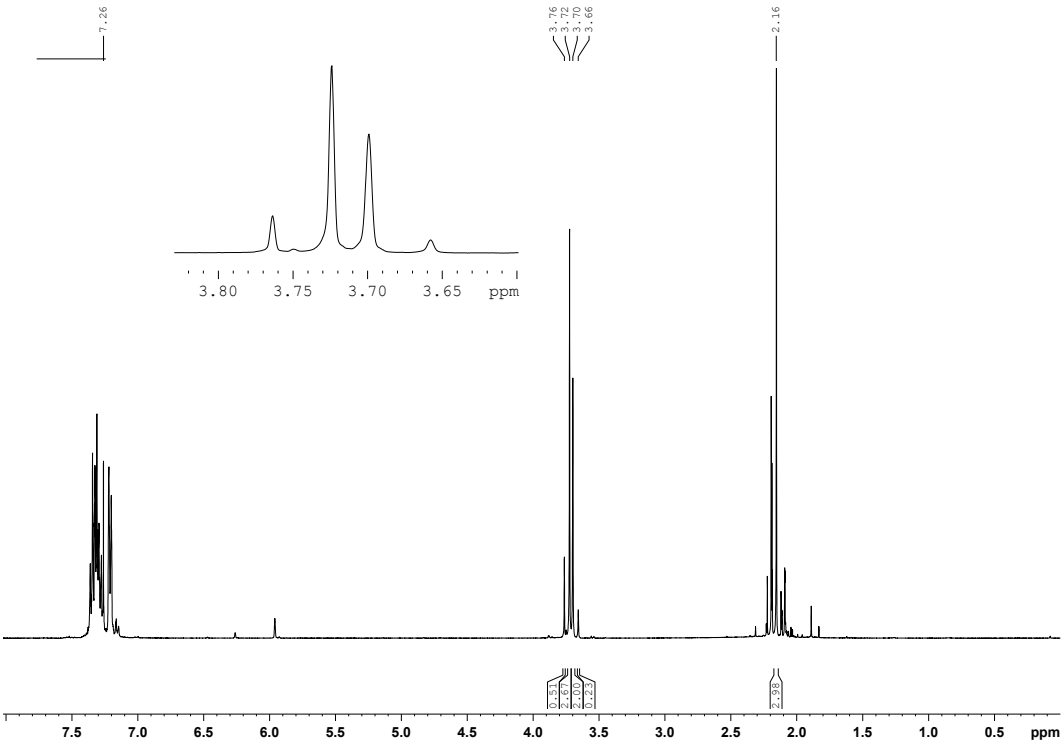


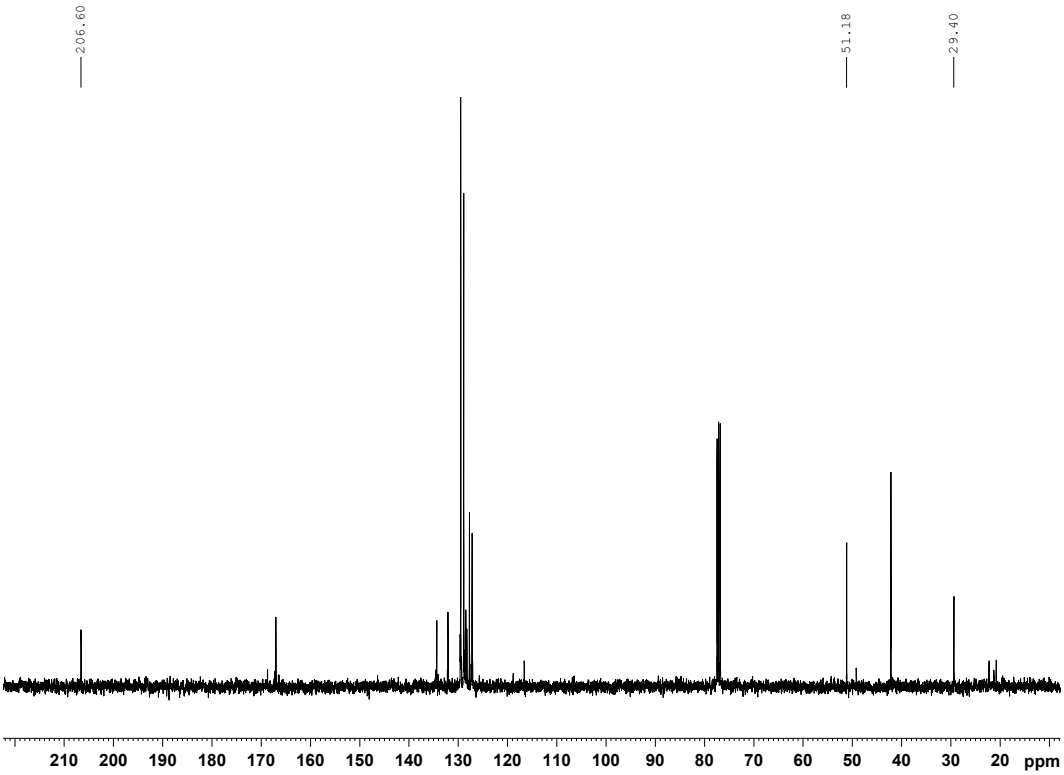


P2P 5 crude from route III ^{1}H NMR spectra

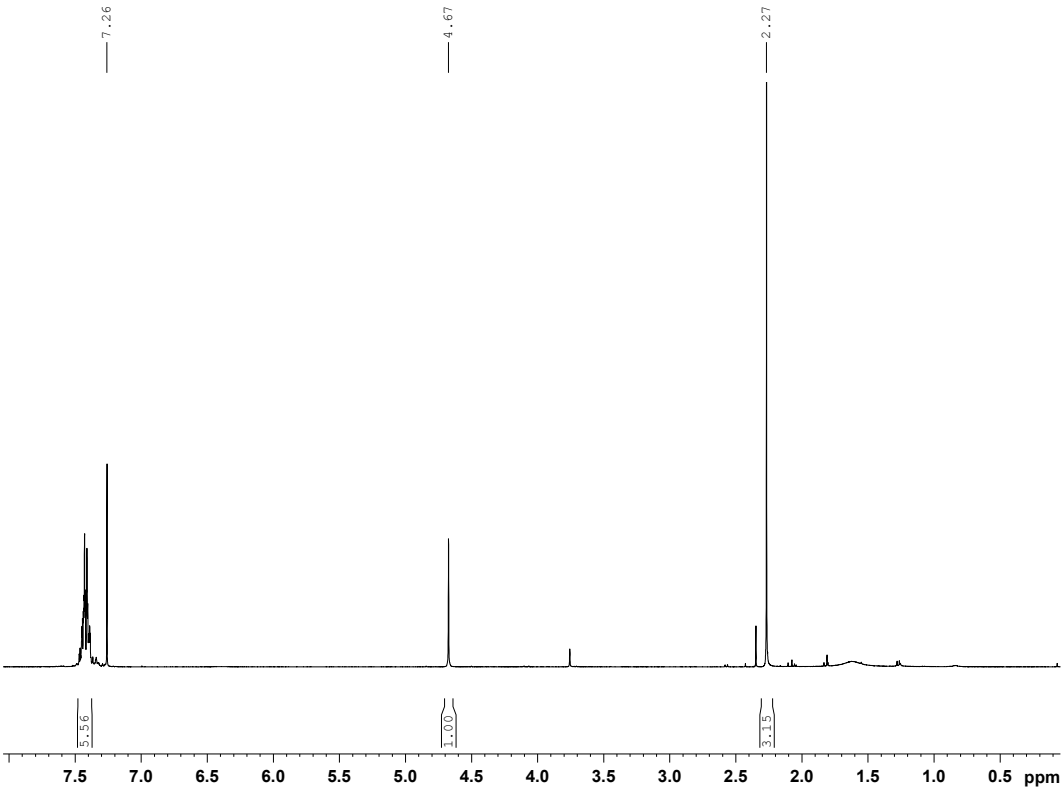


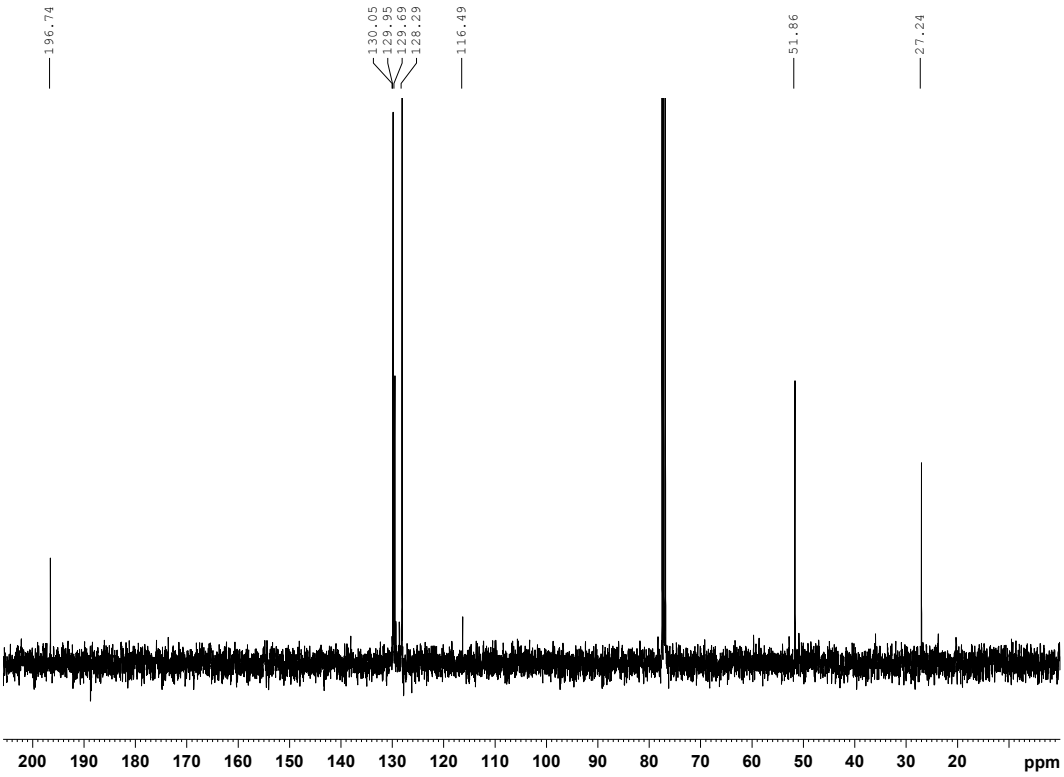
P2P 5 crude from route IV ^{1}H and ^{13}C NMR spectra



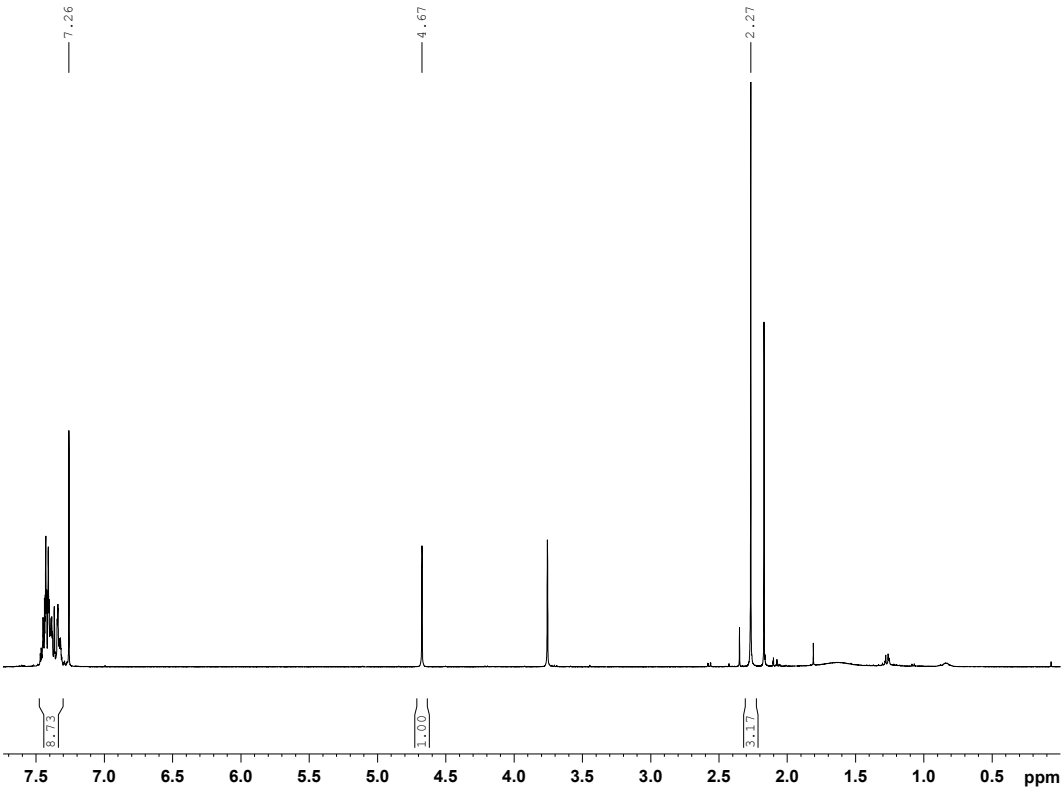


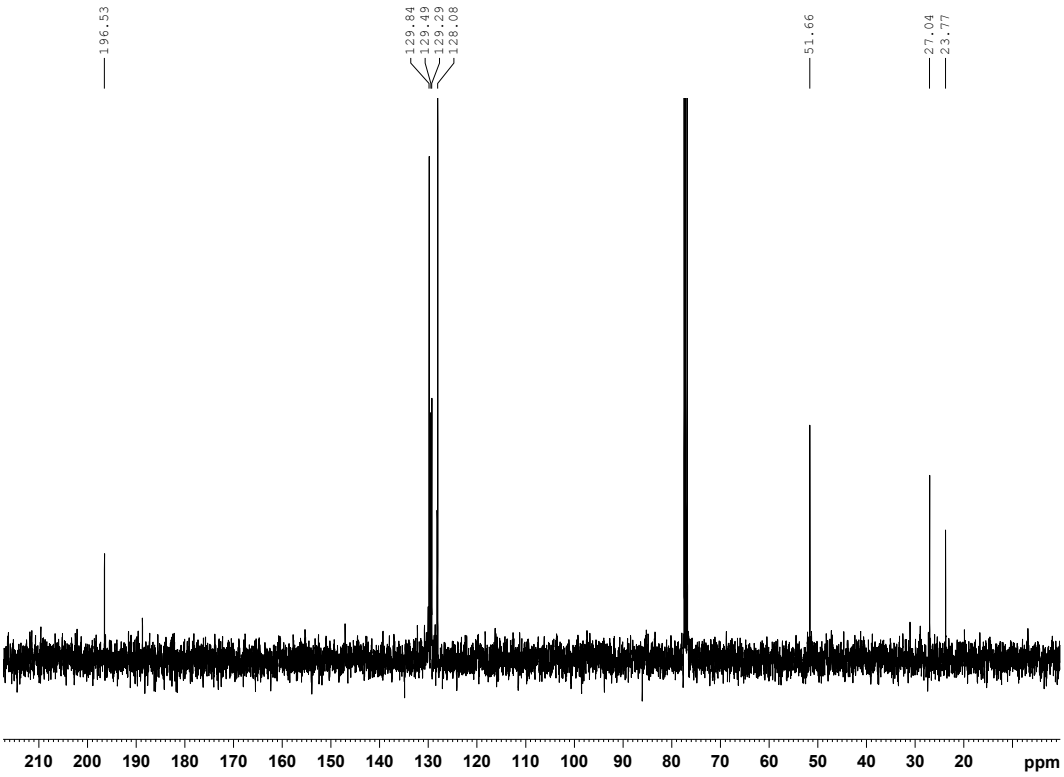
APAAN 10 crude from route I ^{1}H and ^{13}C NMR spectra





APAAN 10 crude from route II ^{1}H and ^{13}C NMR spectra





Appendix 8: The results from the GC-MS analysis

The GC-MS results from the crude amphetamine **1** samples from routes **I** (näyte 1), **I** (näyte 2), **III** (näyte 3) and **IV** (näyte 4), as well as the amphetamine sulfate crystallized from route **II** (näyte 5) and **IV** (näyte 6). **Bolded** substances were found also in the UPLC-TOF-MS anlysis. The shaded substances were the most prominent peaks in the sample. "Nopsa_lib" is the national forensic laboratory's own library and "W9N11" is a commercial library from Wiley.

Näyte 1.	Yhdiste	CAS	rt (min.)	kirjasto	match %
1.	l-valiini (istd)	17609-47-1	1.046	Nopsa_lib.	83
2.	**amfetamiini 1**	300-62-9	1.316	Nopsa_lib.	83
3.	4-methyl-5-phenylpyrimimidine	57562-58-0	2.239	W9N11	97
4.	**Acetamide** ***N-Acetylamphetamine* 13**	14383-60-9	2.330	W9N11 *2015ENFSI*	95 *97*
5.	4,6-dimethoxy-5-methylphthalide	998202-93-6	2.762	W9N11	80
6.	2,4-hexanedione	32588-40-2	2.824	W9N11	64
7.	1,1-diphenyl-1-butene	1726-14-3	2.857	W9N11	64
8.	1,3-diphenyl-3-methyl-1-butene	7614-93-9	2.948	W9N11	91
9.	N-(-phenylisopropyl) benzaldimine	998687-17-0	2.996	W9N11	91
10.	5H-Dibenzo[a,d]cycloheptene, 10,11-dihydro-10,11-dimethyl-, (10R-trans)-	115474-54-9	3.255	W9N11	89
11.	di-(-phelisopropyl)amine)	998333-41-2	3.321	W9N11	87

12.	1-benzyl-3-methylnaphthalene	998273-42-8	3.665	W9N11	99
13.	1,3-dimethyl-2-phenyl-naphthalene	998273-40-9	3.733	W9N11	99
14.	5-alfa-kolestaani (istd)	481-21-0	5.122	Nopsa_lib.	99
Näyte 2.	Yhdiste	CAS	rt (min.)	kirjasto	match %
1.	l-valiini (istd)	17609-47-1	1.045	Nopsa_lib.	83
2.	Benzenemethanol	100-51-6	1.100	W9N11	98
3.	**amfetamiini 1**	300-62-9	1.332	Nopsa_lib.	90
4.	**1-fenyyli-2-propanoli 16**	698-87-3	1.342	Nopsa_lib.	95
5.	Benzeneethanamine	457-87-4	1.591	W9N11	90
6.	Methylphenidate	113-45-1	1.650	W9N11	64
7.	Naphtalene	581-40-8	2.114	W9N11	96
8.	**Acetamide 13**	143383-60-9	2.329	W9N11	92
9.	1,1-diphenyl-1-butene	1726-14-3	2.761	W9N11	86
10.	1,3-diphenyl-3-methyl-1-butene	7614-93-9	2.948	W9N11	89
11.	N-(-phenylisopropyl) benzaldimine	998687-17-0	2.995	W9N11	91
12.	Benzylamphetamine	57378-23-1	3.104	W9N11	59
13.	di-(-phelisopropyl)amine)	998333-41-2	3.350	W9N11	87
14.	1-benzyl-3-methylnaphthalene	998273-42-8	3.661	W9N11	99
15.	1-(2’,6’-Dimethylphenyl)naphthalene	998273-39-6	3.727	W9N11	95

16.	3-(6-Ethoxypyridin-3-yl)quinoline)	998324-83-2	3.753	W9N11	90
17.	p,α-Dimethylstyrene	1195-32-0	3.847	W9N11	64
18.	3,4-Dihydro-1(2H)-naphthalenone	529-34-0	4.412	W9N11	81
19.	5-alfa-kolestaani (istd)	481-21-0	5.120	Nopsa_lib.	99

Näyte 3.	Yhdiste	CAS	rt (min.)	kirjasto	match %
1.	l-valiini (istd)	17609-47-1	1.044	Nopsa_lib.	83
2.	**amfetamiini**	300-62-9	1.318	Nopsa_lib.	83
3.	4-Phenyl-3-buten-2-one	1896-62-4	1.911	W9N11	98
4.	4-Methyl-5-phenylpyrimidine	57562-58-0	2.239	W9N11	97
5.	N-formylamphetamine	15302-18-8	2.297	W9N11	93
6.	5-Benzylpyridin-2-amine	98477-40-8	2.309	W9N11	83
7.	**Acetamide** ***N-Acetylamphetamine*** **13**	14383-60-9	2.335	W9N11 *2015ENFSI*	95 *96*
8.	1(2H)-Phenanthrenone	62264-34-0	2.416	W9N11	83
9.	2,2-Diphenylpropane	778-22-3	2.963	W9N11	86
10.	N-(-phenylisopropyl) benzaldimine	998687-17-0	2.997	W9N11	91
11.	Benzylphenethylamine *dibentsyylimetanamiini*	998213-06-1 -	3.106	W9N11 Nopsa_lib.	72 72

12.	di-(-phelisopropyl)amine)	998333-41-2	3.322	W9N11	87
13.	1-oxo-1-phenyl-2-(-phenylisopropylimino) propane	998367-56-6	3.564	W9N11	91
14.	Isobutyrophenone	611-70-1	3.691	W9N1172	72
15.	1,3-dimethyl-2-phenyl-naphthalene	998273-40-9	3.703	W9N11	99
16.	1-Cyano-2-methoxy-5,5-diphenyl-1,3-cyclopentadiene	998390-91-0	3.741	W9N11	83
17.	trans-2-methoxycarbonyl-3-phenylaziridine	998123-65-4	3.761	W9N11	64
18.	2,6-Dimethyl-3,4,5-triphenylpyridine	998554-83-9	4.471	W9N11	87
19.	N,N'-dicyano-2-(4pyridyl)pyrazino[2,3-b]naphthoquinodiimine	998554-08-6	4.805	W9N11	83
20.	2,4-Dimethyl-3,5,6-triphenylpyridine	998554-83-8	4.865	W9N11	86
21.	5-alfa-kolestaani (istd)	481-21-0	5.120	Nopsa_lib.	99
Näyte 4.	Yhdiste	CAS	rt (min.)	kirjasto	match %
1.	l-valiini (istd)	17609-47-1	1.043	Nopsa_lib.	83
2.	Benzenemethanol	100-51-6	1.091	W9N11	98
3.	**amfetamiini**	300-62-9	1.319	Nopsa_lib.	78
4.	(1R)-N-Ethyl-1-methyl-2-phenylethanamine	33817-12-8	1.589	W9N11	86
5.	2-Phenylacetamide	103-81-1	2.022	W9N11	96
6.	**Acetamide 13**	143383-60-9	2.334	W9N11	95

7.	N-(-phenylisopropyl) benzaldimine	998687-17-0	2.999	W9N11	91
8.	**1,3-Diphenyl-2-propanone 6**	102-04-5	3.013	W9N11	95
9.	3-amino-4-phenylbutan-2-one	40513-35-7	3.110	W9N11	64
10.	3,4-diphenyl-3-buten-2-one	998245-28-4	3.192	W9N11	96
11.	di-(-phelisopropyl)amine)	998333-41-2	3.336	W9N11	87
12.	(1-Ethylpropyl)benzene	1196-58-3	3.356	W9N11	53
13.	1-oxo-1-phenyl-2-(-phenylisopropylimino) propane	998367-56-6	3.565	W9N11	91
14.	Ethyl 2-(benzoylamino) propanoate	998239-78-9	3.686	W9N11	64
15.	2,3-Dimethyl-2,3-diphenylbutane *dicumene*	1889-67-4	4.636	W9N11	64
16.	5-alfa-kolestaani (istd)	481-21-0	5.121	Nopsa_lib.	99

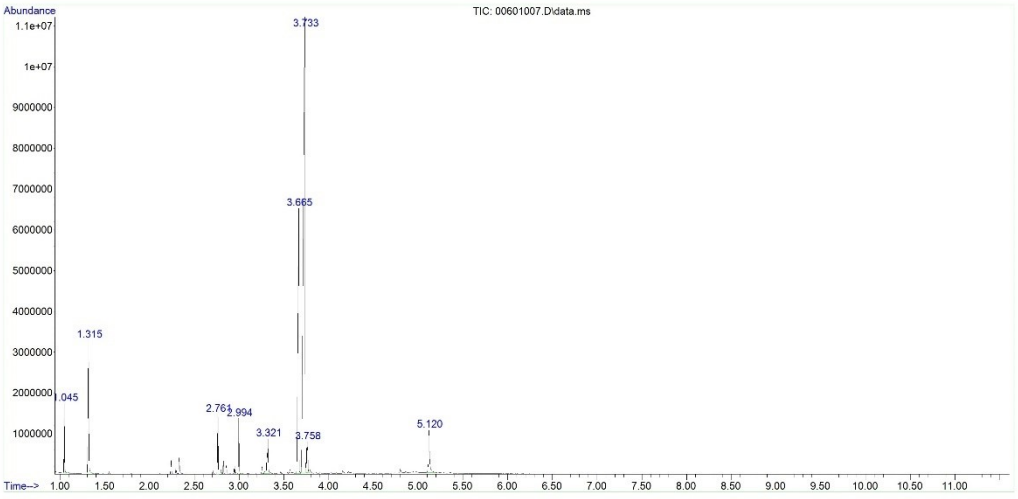
Näyte 5.	Yhdiste	CAS	rt (min.)	kirjasto	match %
1.	l-valiini (istd)	17609-47-1	1.045	Nopsa_lib.	83
2.	amfetamiini	300-62-9	1.324	Nopsa_lib.	83
3.	di-(-phelisopropyl)amine)	998333-41-2	3.321	W9N11	87
4.	1,3-dimethyl-2-phenyl-naphthalene	998273-40-9	3.699	W9N11	99
5.	5-alfa-kolestaani (istd)	481-21-0	5.120	Nopsa_lib.	99

Näyte 6.	Yhdiste	CAS	rt (min.)	kirjasto	match %
1.	l-valiini (istd)	17609-47-1	1.045	Nopsa_lib.	83
2.	amfetamiini	300-62-9	1.323	Nopsa_lib.	83
3.	Benzylphenethylamine *dibentsyylimetanamiini*	998213-06-1 -	3.106	W9N11 Nopsa_lib.	91 90
4.	5-alfa-kolestaani (istd)	481-21-0	5.121	Nopsa_lib.	99

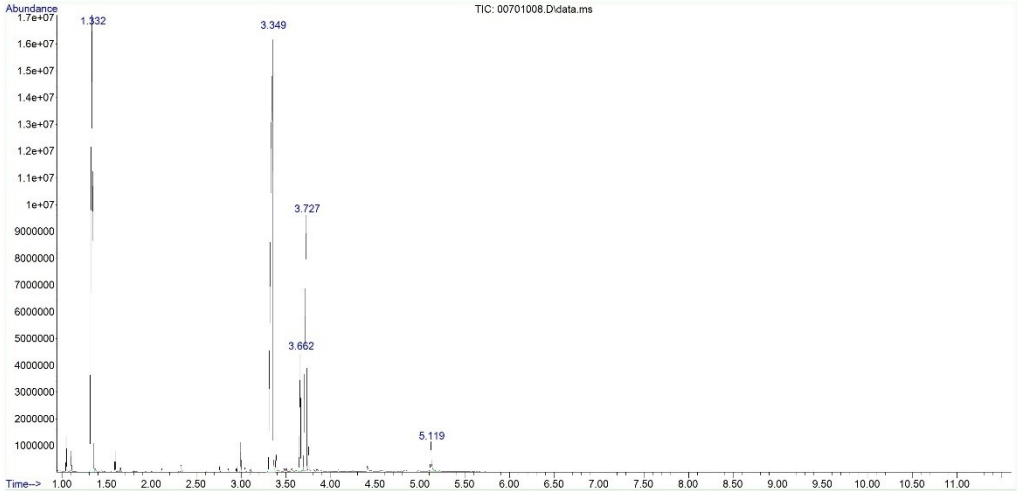
Appendix 9: GC-MS chromatograms

The GC-MS chromatograms from the crude amphetamine **1** oil samples from routes **I** (näyte 1), **I** (näyte 2), **III** (näyte 3) and **IV** (näyte 4), as well as the amphetamine sulfate crystallized from route **II** (näyte 5) and **IV** (näyte 6).

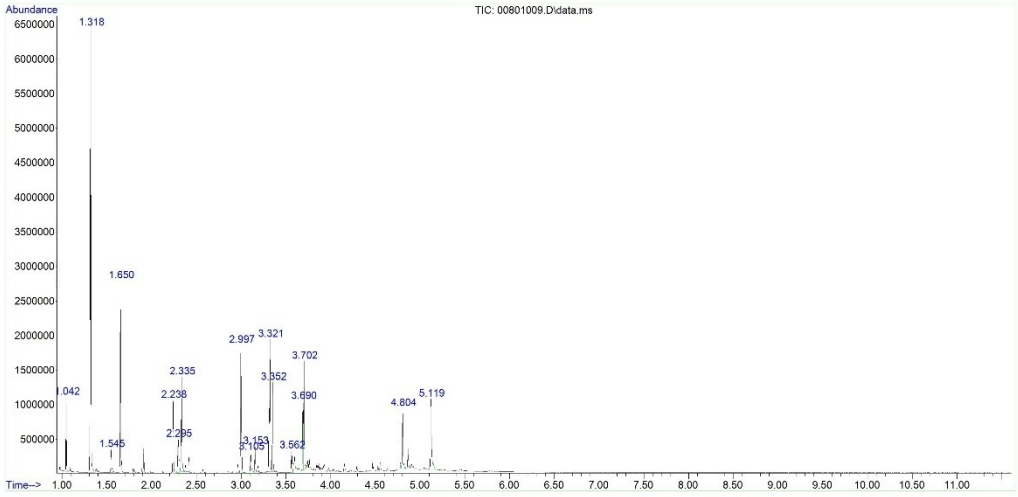
“Näyte 1”:



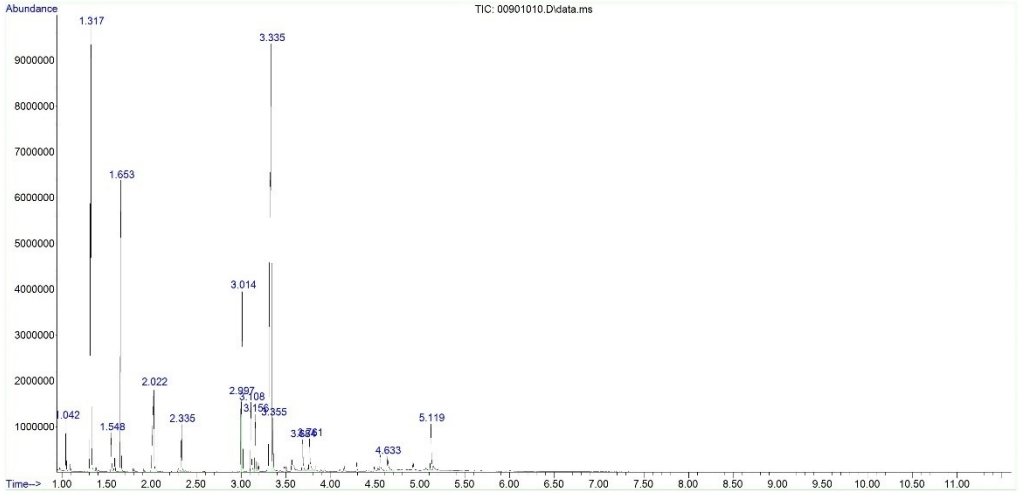
“Näyte 2”:



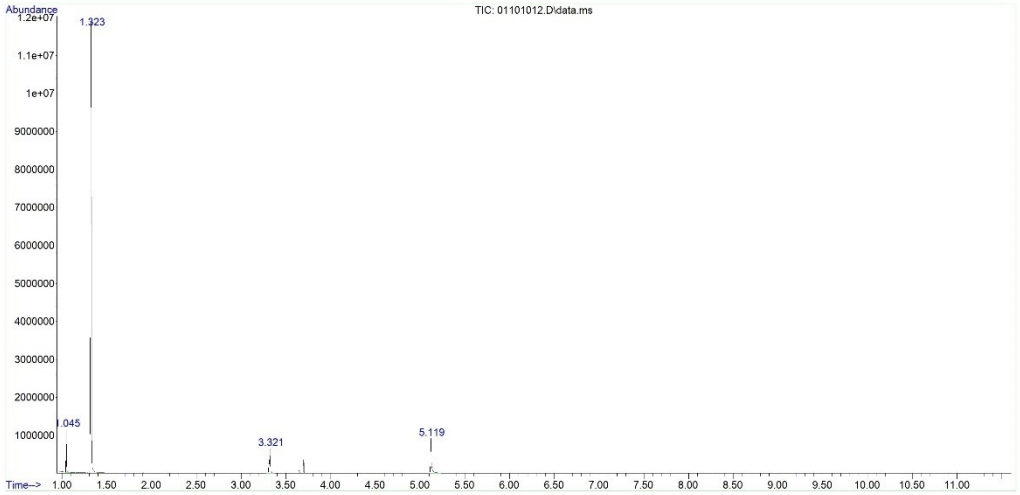
“Näyte 3”:



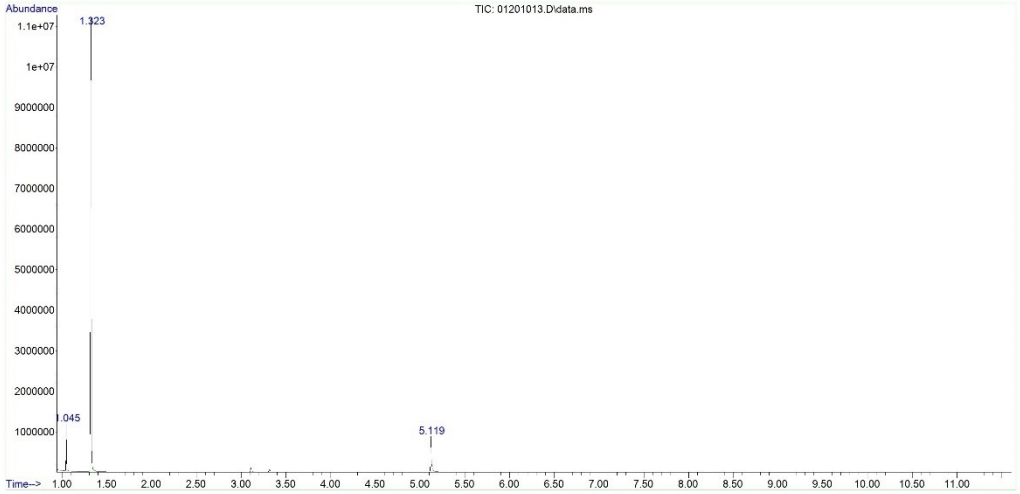
“Näyte 4”:



“Näyte 5”:



“Näyte 6”:


Abundance
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